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Symposium on

The Role of Some of the Newer Vitamins in Human Metabolism and Nutrition*

VANDERBILT University School of Medicine welcomes this group of scientists. The fact that such a distinguished group of speakers has agreed to participate in this symposium and the large out-of-town attendance indicate the wide interest in the topics to be discussed. All of us are indebted to the National Vitamin Foundation which has made this symposium possible. Those of us directly concerned with the symposium are particularly indebted to Doctor Robert S. Goodhart, Scientific Director of the National Vitamin Foundation, and to his office for their cordial assistance in so many ways in the organization of the meeting.

Each species has its own particular vitamin-requirement spectrum. The dietary requirement spectrum for man ranges from the obvious to the occult—from the clear dietary dependence for ascorbic acid to the less defined need for nutrients such as pantothenic acid. In at least the instance of vitamin K, the nutrient plays an essential role in man, but the evidence indicates that he is not dependent upon the diet for a supply of this vitamin. This nutrient is produced by gastrointestinal synthesis. Several species of animals possess an ability to synthesize one or another vitamin in varying amounts—thus the rat can make both niacin and ascorbic acid in quantity sufficient for its needs, but requires niacin if the dietary supply of specific precursors is limited. Where man is known to synthesize vitamins, the synthesis is from specific precursors—vitamin A from carotene, niacin from tryptophan.

One may identify three important broad levels of study of a vitamin in man: (1) *dietary deficiency*, whereby the syndrome resulting from withdrawal of a factor from the diet of a dependent species is investigated;

* Held at Vanderbilt University, Nashville, Tennessee, in co-operation with the National Vitamin Foundation, October 20–21, 1955.

(2) *non-dietary deficiency*, in which the syndrome is studied which results from a deficiency of an essential "metabolic nutrient" which, under usual circumstances, is made either in the gut or tissues of man in quantity to meet the physiologic needs; and (3) *metabolic deficiency*, in which a syndrome or effect of a deficient quantity of the vitamin at the tissue level is studied. "Metabolic deficiency" does not depend upon the factor being a dietary essential. While the metabolic deficiency may be induced by dietary lack, it may result also from removal or blockage of a synthetic process or by interference with a key metabolic reaction in which the vitamin (or its derivative) plays some role. Studies at this level, such as those employing antivitamins or antimetabolites, give important information as to whether a vitamin is an *essential metabolite* for a species. If such studies indicate that a vitamin is an essential metabolite, the other two levels of study provide the complete nutritional picture of the factor.

In keeping with these thoughts, we have arranged this symposium on the role of some of the newer vitamins in human metabolism and nutrition. It is our hope that it will bring up to date the evidence bearing on the roles in human nutrition of vitamin B_6, tocopherol, and pantothenic acid.

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In Memoriam / DR. JAMES FLEECE RINEHART, 1901–1955

A distinguished career was abruptly terminated at San Mateo, California, on November 30, 1955 at the early age of 54 with the sudden passing of Dr. James Fleece Rinehart, Professor of Pathology at the University of California School of Medicine in San Francisco.

Dr. J. F. Rinehart.

Dr. Rinehart was born in Oakland, California, in 1901, attended grammar and high school (Fremont) in that city, graduating from the latter in 1919. With the exception of one year (1920–1921) which was spent at Centre College, Kentucky, he pursued his premedical (1919–1922) and medical education (1922–1926) at the University of California at Berkeley and at its School of Medicine in San Francisco. After serving a rotating internship (1926–1927) at Alameda County Hospital in Oakland, Dr.

Rinehart returned to the University of California School of Medicine in 1927 as assistant in Pathology. Save for the year 1930–1931 when he was Littauer Research Fellow in the Thorndike Laboratory of Harvard University at Boston, he was associated with the University of California until his death. During this short tenure at Harvard Medical School, his contact with Dr. George R. Minot made a marked impression upon him and had considerable influence upon his later thinking and upon his research in the field of nutrition.

Dr. Rinehart early displayed his ability as a pathologist and as an investigator in experimental pathology and nutrition. He advanced rapidly to instructor (1929–1930) and to assistant professor, then to associate professor (1936–1942). Following the death in 1941 of the late Professor of Pathology, Dr. Charles Connor, he was appointed to the position of Chairman of the Department and received the promotion to Professor in 1942, a position which he occupied the remainder of his life. His eminence as a pathologist is attested to by the numerous appointments and positions which he held inside and outside the University. These include: Pathologist, University of California Hospital; Visiting Pathologist, Laguna Honda Home; Member of State Board of Health; Consulting Pathologist for Langley Porter Clinic, Mt. Zion Hospital, Veterans' Administration Hospital and for the Maimonides Health Center for the Chronic Sick. In addition, he served on many academic committees and played an important role in the development of the campus and in the attainment of the new Herbert Moffitt Hospital and Medical Sciences Building. He was a member of numerous medical and scientific societies, served as vice-president (1949) and president (1950) of the American Society for Experimental Pathology, and on the Council of the A.A.A.S. (1954–1955).

The late Professor Rinehart had tremendous drive, a wide and varied interest in science and medicine, and a love for his work. In addition to his role in training of a vast number of medical students and specialists in pathology, he was the author or co-author of some 90 publications dealing with such subjects as: the relationship of vitamin C deficiency to the development of rheumatic fever, the metabolism of vitamin C in rheumatic fever and rheumatoid arthritis, the effect of the bioflavonoids on rheumatic fever, the pathology and biochemistry of experimental vitamin C and B-vitamin deficiencies in the monkey, electron-microscopy of normal and abnormal tissues, and many other aspects of pathology and medicine. He probably will be best remembered for his work* on the pathogenesis of rheumatic fever and of arteriosclerosis.

Dr. Rinehart was filled with kindness and compassion for those who were less fortunate than he. Those who knew him or had been associated with him will always remember his many acts of kindness and his unselfish devotion to the interests of the University of California School of Medicine. He was a champion of the underdog, of the medical student, and of his subordinate personnel. It is believed that he made his demise as he would have liked: he died "with his boots on."

—LOUIS D. GREENBERG

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Vitamin B_6 Deficiency in the Rhesus Monkey with Particular Reference to the Occurrence of Atherosclerosis, Dental Caries, and Hepatic Cirrhosis. Rinehart, J. F., and Greenberg, L. D. AM. J. CLIN. NUTRITION (this issue, page 318).

The History of Vitamin B_6

By PAUL GYÖRGY, M.D.*

HISTORICAL reminiscence implies for the participant of the event in question the interplay of unavoidable, subjective, personal considerations. It is difficult, well-nigh impossible, for the active participant to observe a completely detached, objective attitude. On the other hand, it is equally dangerous for anybody not having been actively engaged in the particular historical development to reconstruct *a posteriori* the events as they occurred.

The discovery of vitamin B_6 has to be reconstructed as in any historical process, in the light of knowledge available at the time of the discovery, and not with convenient "hindsight." It has been part of one of the most intriguing chapters in the rapid development of vitamin research: the unravelling of the vitamin B complex.

By the end of 1932 only two separate components of the vitamin B complex had been distinguished as needed by the rat: (a) vitamin B_1, the antineuritic factor; and (b) vitamin B_2 (or vitamin G), the antipellagra factor. The separate existence of a third factor called vitamin B_4, absence of which was said[1,2] to be associated with symptoms of nerve lesions, such as disturbances of co-ordination and ataxia, had not been generally accepted. Even less credit had been given to the claim that there were two more special factors, B_3 and B_5, as needed by pigeons.[3] Chick and Copping[4] had postulated the existence of a separate growth-promoting factor, called by them Factor Y, but neither its specific biologic effect nor its relation to the vitamin B complex had been investigated.

The British Committee on Accessory Food Factors[5] in 1927 defined vitamin B_2 as "the more heat stable, water-soluble dietary factor, recently described and named P-P ("pellagra-preventive") factor by Goldberger, Wheeler, Lillie, and Rogers (1926) and found necessary for maintenance of growth and health and prevention of characteristic skin lesions in rats, and considered by the latter workers to be concerned in the prevention of human pellagra."

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EARLY STUDIES

The first way-station on the road to the solution of the puzzling vitamin B complex was reached by the isolation of riboflavin, originally obtained in 1933 from milk and called lactoflavin, as the result of a co-operative study of Richard Kuhn, Th. Wagner-Jauregg, and myself.

For the production of vitamin B_2 deficiency in rats, we first used a ration originally devised by Bourquin and Sherman[6] which employed an alcoholic extract of wheat as the source of vitamin B_1. Pure vitamin B_1 was not available at that time. On the diet of Bourquin and Sherman, the weight curves of young rats soon flattened out or showed a decline. Addition of crude extracts of yeast, rice bran, liver, or of milk concentrates (or whey), in all of which vitamin B_1 was destroyed by autoclaving, restored normal growth.[7]

Modern research workers, accustomed to microbiologic tests which give an answer in 24 to 40 hours, and which permit the simultaneous assays of scores of test substances, should be impressed by the fact that each assay for vitamin B_2 in rats required a testing period of three to four weeks, with a corresponding number of prepared experimental animals.

Doctor Wagner-Jauregg first noted that all concentrates which proved to be active in the animal assays were colored and showed an intensive green-yellow fluorescence, in direct proportion to their biological effect. Exposure to visible light destroyed the growth-promoting activity of these concentrates.[8] The obvious working hypothesis, which identified vitamin

B_2 with a yellow-green pigment, soon met serious difficulties when concentrates that were further purified and more highly colored proved to be biologically inactive in the rat growth test. Here the biologist, or animal experimentalist, came to the rescue of the chemist. It was shown that by supplementing the diet with a yeast concentrate from which all colored material had been removed by adsorption, the biologic activity of the colored preparation was restored. Thus, it was proved that vitamin B_2 is not a single substance but that it may be separated into at least two components, one of which was characteristically a pigment: riboflavin.

The question arose whether riboflavin, which to some extent fell under the definition of vitamin B_2 as given above, had any relation to human pellagra, or to its experimental counterpart, canine black-tongue. In co-operation with Birch and L. J. Harris,[9] we were able to show, independent of and simultaneously with the Wisconsin group under Elvehjem,[10] that riboflavin was different from the specific pellagra-preventive factor (P-P) of Goldberger and his associates. Elvehjem and his colleagues[11] later identified nicotinic acid (amide) with the antipellagra factor, adding another chapter to the fascinating story of the unraveling of the vitamin B_2 complex. Inasmuch as riboflavin was the first member of the vitamin B_2 complex isolated and identified, it is not surprising that it is still often called vitamin B_2, without reference to the comprehensive character of the original term "vitamin B_2."

ACRODYNIA

A satisfactory supplement to promote further growth in rats receiving pure, crystalline vitamin B_1 and riboflavin was found to be the so-called vitamin B_4 concentrate obtained according to Peters and his collaborators[1,2] by adsorption of a yeast concentrate on charcoal and subsequent elution with alcohol acidified with hydrochloric acid. In young rats kept on a semi-synthetic diet with added vitamin B_1 and riboflavin, but without any further supplement, severe cutaneous lesions soon developed, which were characterized by edema, redness, scaliness of the paws, snout, nose, and ears, i.e., of the most distal parts of the body. Correspondingly we called the condition rat acrodynia without any prejudice as to whether this condition is in its causation and pathogenesis analogous to a human condition.

As a matter of fact, at present it may be stated without any reservation that the usual human acrodynia is not based on a deficiency of any constituent of the vitamin B_2 complex. On the other hand, during the starvation period in Italy after the recent war, Professor Frontali (Rome) observed young infants with typical cutaneous manifestations which responded to treatment with the same factor of the vitamin B_2 complex which prevents and cures rat acrodynia.

The dermatologic condition of rat acrodynia as a state of specific dietary deficiency has not been described previously. The yeast concentrate of the so-called vitamin B_4 factor, prepared according to Peters and his associates,[1,2] had in our experience a definite curative and preventive effect on rat acrodynia. Inasmuch as vitamin B_4 deficiency was supposedly characterized exclusively by nerve lesions,[2] rat acrodynia must have been due to the deficiency of another constituent of yeast, also present in the yeast concentrate of Peters and his associates. The even less well defined factors, B_3 and B_5, claimed to be required by the pigeon, have been disregarded and the factor curing rat acrodynia has been named vitamin B_6[12] and as such delineated from all other constituents of the vitamin B_2 complex.

It may be added that later Peters and his associates[13] revoked the existence of vitamin B_4 and explained their previous observations on this deficiency by simultaneous hypovitaminosis of vitamin B_1 and riboflavin.

The clinical picture of experimental vitamin B_6 deficiency changed according to species studied, as well as the age of the animals and also to extraneous dietary conditions. In our original studies, we relied chiefly on the cutaneous manifestations as the easily recognizable, specific sign of vitamin B_6 deficiency. In collaboration with Birch,[14] we further observed that fat with a high percentage of unsaturated fatty acids, at that time also called vitamin F, prevented and beneficially influenced these

cutaneous manifestations of vitamin B_6 deficiency. Later Birch[15] advanced the idea that the essential fatty acids were necessary for the utilization of vitamin B_6 and, in return, vitamin B_6 was required for the utilization of the essential fatty acids.

Among other manifestations of vitamin B_6 deficiency, it was early recognized that in young animals, such as rats, pigs,[16–18] and dogs [19] epileptiform convulsions may appear. Recently these experimental observations had led to the recognition of vitamin B_6 deficiency in young infants, as will be discussed later.

In dogs[20] and in pigs[16,21] lacking vitamin B_6, a microcytic anemia developed. It has been claimed[22] that in rhesus monkeys chronic vitamin B_6 deficiency leads to arteriosclerotic changes.

All these abnormal conditions responded promptly and specifically to vitamin B_6 medication. In many instances vitamin B_6 deficiency in animals became apparent only by nonspecific retardation of growth, and most authors used the retardation of growth as the criterion of vitamin B_6 deficiency. Under well-controlled experimental conditions the growth assay has proved to be useful, in spite of its unspecific character. It deserves to be pointed out that in the experimental animal vitamin B_6 added to a semisynthetic diet supplemented only with vitamin B_1 and riboflavin will prevent or cure the specific acrodynia and the epileptiform convulsions, as well as the microcytic anemia, but will not promote growth, or—in curative experiments—only for a very short period, after which the growth will again decline. This observation led Jukes and Lepkovsky[23] to the recognition of pantothenic acid, first called "filtrate factor," having been found in the filtrate of yeast extract after charcoal adsorption. The addition of pantothenic acid is required for the promotion of growth, in addition to vitamin B_1, riboflavin, and vitamin B_6. Thus, another member has been added to the rapidly increasing number of constituents of the vitamin B_2 complex.

PYRIDOXINE

The chemical nature of vitamin B_6 has been studied in collaboration with Birch[15] on crude concentrates of the vitamin. The isolation of pure crystalline vitamin B_6 was first reported by Lepkovsky[24] in 1938, barely four years after recognition of this specific member of the vitamin B_2 complex. Independently, but slightly later, several other groups[25–28] also reported the isolation of vitamin B_6. Within a year Harris and Folkers[29] and Kuhn with his associates[30] showed that vitamin B_6 was a pyridine derivative, specifically 3-hydroxyl-4, 5-dihydroxymethyl-2-methyl-pyridine. The term *pyridoxine*, proposed by us[31] for this compound has received general acceptance.

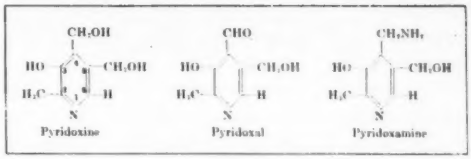

Figure 1

At this stage of the historical development, microbiological research entered the scene. Credit is due to Snell and his associates[32] for first recognizing the existence of other forms of pyridoxine as the result of the comparison of microbiologic assays on extracts of natural materials with the values based on chemical and animal assay. Snell[33,34] further observed that autoclaving of pyridoxine with the assay medium or amino acids greatly increased the activity of pyridoxine for the test organism *Streptococcus faecalis R*. This increased activity was assigned to the aminated product of pyridoxine called pyridoxamine. Through mild oxidation the formyl derivative of pyridoxine, pyridoxal, was obtained (Fig. 1).

Pyridoxine, pyridoxal, and pyridoxamine may occur naturally in free or in several conjugated or "bound" forms.[35] The latter include pyridoxal phosphate and pyridoxamine phosphate and their combinations with protein and perhaps other unidentified conjugates. The fact that pyridoxine occurs in bound form in many natural products has also been observed by Birch and myself.[14]

Pyridoxal, pyridoxamine, and pyridoxine are as a rule equal in activity for animals[36,37]; in many instances, however, pyridoxal and pyridoxamine show slightly less activity than

pyridoxine. In contrast, for many micro-organisms the three forms show very different activities.[36]

In natural food products, pyridoxal and pyridoxamine and the corresponding conjugates are in excess of pyridoxine. Pyridoxine is more resistant to heat than the other natural forms of vitamin B_6.[38,39] This observation recently became of great practical significance, in relation to the convulsive disorders seen in infants receiving autoclaved liquid milk preparations, as will be discussed later in detail during this Symposium.

It became customary[40] to speak of vitamin B_6 as a sub-*group* of the vitamin B_2 complex, with pyridoxine, pyridoxal, pydridoxamine, etc. as its particular chemical representatives.

FUNCTION OF VITAMIN B_6

A large number of investigations and publications in the years closely following the discovery of vitamin B_6 were concerned with the metabolic activity of this vitamin, both *in vivo* and *in vitro*. As first shown for riboflavin[41] and later confirmed for all other members of the vitamin B_2 complex, it was to be expected that vitamin B_6 as another member of the vitamin B_2 complex should act not only as a vitamin but also as a proenzyme. Pyridoxine, pyridoxal, pyridoxamine, and their respective phosphates owe their vitamin activity to the ability of the organism to convert them into the enzymatically active form, i.e., pyridoxal-5-phosphate.

Vitamin B_6 was found to participate in a wide variety of enzyme systems, in the intracellular and extracellular metabolic utilization and transformation of amino acids. The most important relevant reactions are decarboxylation and transamination. In consequence, it was to be expected that deficiency of vitamin B_6 would manifest itself metabolically in some aberration of amino acid metabolism. One of the first such pathologic pathways was found in the utilization of tryptophan even in—what may be called—relative vitamin B_6 deficiency. In such conditions, as first shown by Lepkovsky and his associates,[42] an extra load of tryptophan will increase the urinary excretion of xanthurenic acid.

With all this rich history of vitamin B_6, it took approximately 20 years until its requirement by the human organism could be definitely established and recognized. From this point of view, vitamin B_6 is one of the newer vitamins as stated in the title of this symposium. For those of us who are both physicians and experimentalists, the need of man for vitamin B_6 has not come as a surprise. Vitamin B_6 is required by all animals studied and by a very large number of micro-organisms. Further, it has proved to be one of the key constituents of important enzyme systems. Under these circumstances, and in analogy to other similar nutrients, it appeared to be permissible *a priori* to extrapolate, and to extend the vitamin character of vitamin B_6 to man. Be that as it may, for one who witnessed the whole story of vitamin B_6, it is gratifying to say that vitamin B_6 has come of age.

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Vitamin B_6 Deficiency in the Rhesus Monkey

WITH PARTICULAR REFERENCE TO THE OCCURRENCE OF ATHEROSCLEROSIS, DENTAL CARIES, AND HEPATIC CIRRHOSIS

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IN 1949[1] we reported initial observations on a small series of rhesus monkeys subjected to pyridoxine deficiency, calling attention to the occurrence of degenerative vascular lesions. In subsequent reports attention was again called to the essential similarity of the experimental vascular lesions and those of arteriosclerosis in man.[2,3]

Studies of pyridoxine deficiency in the monkey (*Macaca mulatta*) have continued. This report is based upon observations on some 40 animals subjected to acute or chronic pyridoxine deficiency and a considerable number of animals which were given full supplements or subjected to other deficiencies. While these data have not been completely analyzed, the general pattern of the pathologic alterations of pyridoxine deficiency seems clear.

METHOD OF STUDY

The basal diet used in most of the experiments was a modification of the M-3 diet of Waisman and associates[4] and consisted of powdered sucrose 73 per cent, vitamin test casein (Labco) 18 per cent, Hawk & Oser salt mixture 4 per cent, and corn oil 2 per cent. The dry ingredients were thoroughly blended, granulated, and compressed into 2-g tablets after the addition of 1 per cent calcium stearate. These tablets were fed *ad libitum*. Each monkey received one vitamin tablet daily containing the following: thiamine, 0.5 mg; riboflavin, 1 mg; nicotinic acid, 5 mg; calcium pantothenate, 3 mg; ascorbic acid, 25 mg; *p*-aminobenzoic acid, 100 mg; choline dihydrogen citrate, 100 mg; inositol, 100 mg; and biotin, 10 micrograms, plus sufficient powdered sucrose to make a tablet weighing 1.5 g. With rare exceptions, the monkeys accepted these vitamin tablets willingly and consumed them eagerly. Control monkeys also received either 1 mg pyridoxine hydrochloride daily or 3.5 mg twice a week (on a sugar cube), except in certain experiments which were designed to test the effect of larger intakes of the vitamin. In addition, each monkey received by mouth 10 drops of a vitamin A and D concentrate (containing 100,000 units of vitamin A and 10,000 units of vitamin D) and 5 drops of mixed natural tocopherols (containing 340 mg/g) weekly.

The monkeys (*Macaca mulatta*) used in these experiments were purchased from dealers and generally weighed between 1.3 and 3.0 kilograms at the time of arrival at the laboratory. They were housed in individual metal cages in well-ventilated quarters, and the cages were cleansed daily by flushing with water. Accurate account was kept of the daily food consumption of each animal, and observations were made almost daily on the appearance and condition of each. Blood for complete blood counts and for biochemical analyses was drawn usually every two to three weeks.

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OBSERVATIONS

Within two to four weeks of withdrawal of pyridoxine from the diet, the monkeys began to lose weight and generally exhibited a progressive decline in weight throughout the period of deprivation. The range of survival time in uncomplicated pyridoxine deficiency has been 6 to 12 months. (One animal survived 23 months.) Aside from decreased food consumption, loss in weight, and some loss in vigor, the animals showed little change in outward appearance until approximately five to nine months had passed. They then appeared unkempt, sluggish in movements, and showed signs of either hyperirritability or apathy. In addition, they frequently developed some degree of periorbital edema. Most of the animals showed some changes in their fur, but this varied considerably from animal to animal. The alterations consisted of shagginess with loss of luster, thinning of the hair in some monkeys, patches of baldness in others, extensive loss of hair in some, while others exhibited hardly any change in the appearance of the coat except for slight graying.

Anemia was invariably manifest by the second month and was unremitting until the animal either expired or received pyridoxine. This anemia was characterized by mild hypochromia, increase in mean cell diameter, decrease in mean cell thickness, the appearance of "target" cells, and increased resistance to hemolysis.[5] The total leukocyte count dropped in 50 per cent of the animals which had been deficient for an average of 13 months. The fall affected the granulocytes and mononuclear leukocytes proportionately without selective lymphopenia, as reported by others.[6]

One experiment was designed to test the effect of graded dosage of pyridoxine on growth, blood transaminase, and development of vascular lesions. This study included 11 young immature monkeys which had been maintained for approximately two months on the basal diet with complete supplements, followed by withdrawal of vitamin B_6 from the diet for a period of 36 to 100 days in order to bring about depletion of the vitamin B_6 stores in these animals. They were then divided into four groups of two animals each and one group of three. Each of the five groups was then supplied with one of the following daily dosages of pyridoxine hydrochloride: 50, 100, 150, 200, and 1000 μg. The group containing three animals received the 1-mg dose. After approximately 60 days on these supplements, the law of diminishing returns was applied to the pyridoxine requirement. When the rate of gain (average daily weight gain) for each group* was plotted against the log of the daily dose of pyridoxine hydrochloride (Fig. 1), the

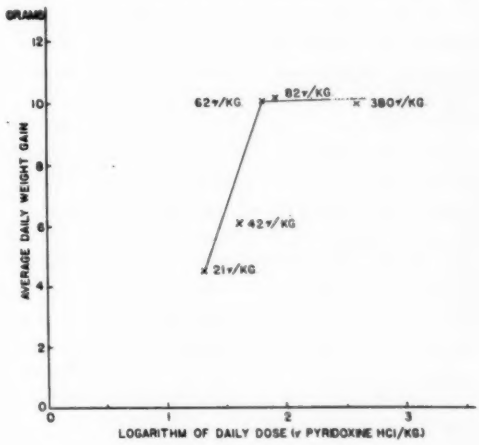

Fig. 1. Pyridoxine requirement for growth.

response was approximately linear for the first three levels and reached a plateau at approximately 62 μg/kg body weight per day. Although the number of animals in each group is small, this value is interpreted as representing the probable pyridoxine requirement for growth in the rhesus monkey. This estimate comes close to the requirement reported for the dog[7] and for the pig.[8]

A study was likewise made of the transaminase and vitamin B_6 levels in the blood of monkeys receiving graded doses of pyridoxine. Significant increases in both levels were observed with increasing intake of pyridoxine. Reductions in pyridoxine intake were followed

* Calculations for the group receiving the 1-mg dose are based upon two animals instead of three, since one of the monkeys became ill during the period under consideration.

by a lowering of the blood concentration of both factors. The changes in the level of transaminase were often more gradual than those of vitamin B_6. An approximate or rough straight line relationship is obtained when the peak transaminase level of a series of monkeys on graded doses of the vitamin is plotted against the log of the daily pyridoxine intake in micrograms per kilogram body weight.[9]

PATHOLOGIC FINDINGS IN PYRIDOXINE DEFICIENCY

Three major pathologic changes develop in pyridoxine deficiency in the rhesus monkey, namely: degenerative changes in arteries, dental caries, and fatty metamorphosis in the liver, often with cirrhosis. The occurrence of each is remarkably constant and holds potentially important implications in human nutrition. The finding of degenerative arterial lesions which most closely simulate those of human arteriosclerosis has been recorded[1,2,3] and need only be presented in brief review. It may be added that such lesions have now been found to be of regular occurrence in a large series of animals studied in our laboratory and in a small series of animals in another laboratory.[10]

Arteriosclerotic Lesions

Any consideration of arteriosclerosis must take into account the mucinous component of the vessel wall which serves not only as a cement substance but permits the free expansile and contractile mobility of the artery. This mucinous component of ground substance is in effect a cement substance, and its integrity is essential for maintenance of the normal structure and function of the arterial wall. This mucinous component is present in the normally delicate intima; it surrounds the lamellae of elastic tissue and lies between the muscle cells, serving as a "mobile cement" holding the cells and fibers of the vessel wall together and allowing them to glide upon one another in the ever-repeated expansion and contraction of the vessel.

In studying the arteries of the pyridoxine-deficient animals, it was soon apparent that the lesions were typified by a swelling of the mucinous ground substance. This is most evident in the intimal zone, but in the more severe lesions there is also a swelling of the ground substance of the media. The intima may become considerably thickened with an increasing accumulation of mucinous material. Concomitantly, there is a proliferation of the cells in the intima. In such lesions fibrillar material, some with the staining properties of collagen and some of the nature of elastic tissue, develops in the mucoid plaque. It seems probable that both the collagenous and elastic tissue fibers are differentiated products derived from the mucinous ground substance.

The sclerotic alterations of arteries are rather widely distributed in the pyridoxine-deficient monkeys. Sites of predilection are: (1) the abdominal, iliac, and femoral arteries; (2) arteries in the testicular tunic; (3) small- and medium-sized branches of the renal arteries. In some degree such lesions are almost constantly observed in these sites. In many animals similar lesions are found in other vessels such as the coronary and mesenteric arteries, and arteries of the pancreas and other viscera.

The distribution of the experimental arteriosclerotic arteries is much the same as that encountered in man, and the morphologic similarities are quite close both in the large and small arteries. It should be recalled that arteriosclerosis in man is a diffusely distributed disease process, although, quite naturally, major attention is focused upon vessels supplying vital structures such as the heart and brain. Characteristic vascular lesions of the vitamin B_6-deficient monkey are shown in Figures 2 to 5. (For a more detailed description of the arteriosclerotic lesions, see references 1 to 3.)

While we do not know the precise amount of pyridoxine that may be required to prevent the development of vascular lesions, it is now clear that suboptimal intakes, experimentally maintained over longer intervals, will result in the evolution of arteriosclerotic lesions of the type described.

To date, a detailed study has not been made of the occurrence of lipid in the experimentally produced arterial lesions. However, lipid has been demonstrable in the more advanced

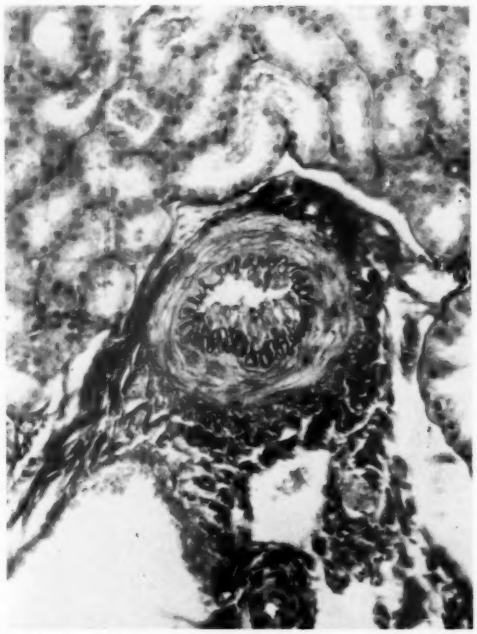

Fig. 2. Intimal fibrosis in a small branch of a renal artery: monkey, 13-month pyridoxine deficiency.

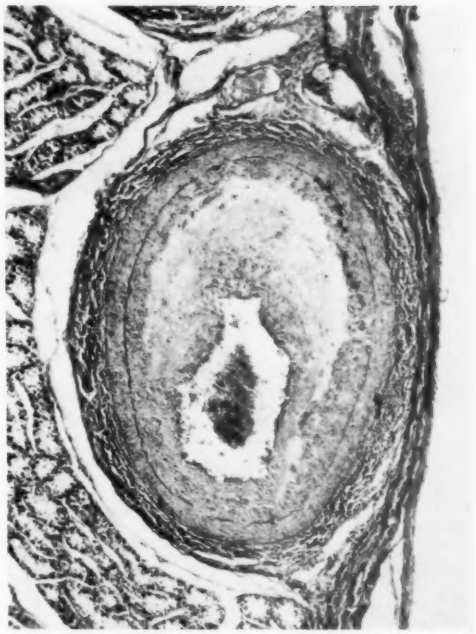

Fig. 3. Marked occlusive intimal fibrosis with delicate edematous appearance of the collagen in an artery of the testicular tunic: monkey, 16-month deficiency.

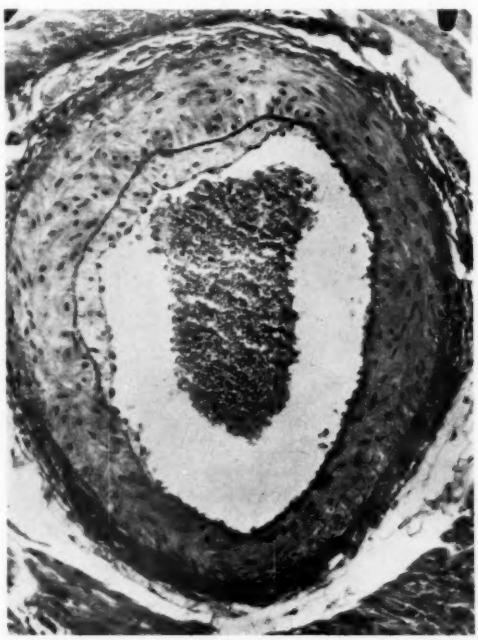

Fig. 4. Early eccentric fibrous plaque in a branch of a coronary artery: monkey, 13-month deficiency.

lesions studied. In some instances it appears that lipid may have combined with the swollen mucopolysaccharide. It has been postulated[11,12] that the mucopolysaccharide may have a positive affinity for lipid.

Fatty Metamorphosis and Cirrhosis of the Liver

A second pathologic alteration of essentially regular occurrence in animals subjected to pyridoxine deficiency has been the development of fatty metamorphosis and, commonly, cirrhosis of the liver. In the experiments of relatively short duration, in which the deficiency state has been maintained for periods of three to eight months, the liver is found to be somewhat enlarged, pale, and yellow, and, on section, the parenchymal cells contain large fat droplets. With more extended periods of deficiency, degrees of scarring, dominantly periportal but sometimes diffuse, become evident. In numerous cases there are relatively large nodules of regenerative hyperplasia, some of which are difficult to differentiate from neo-

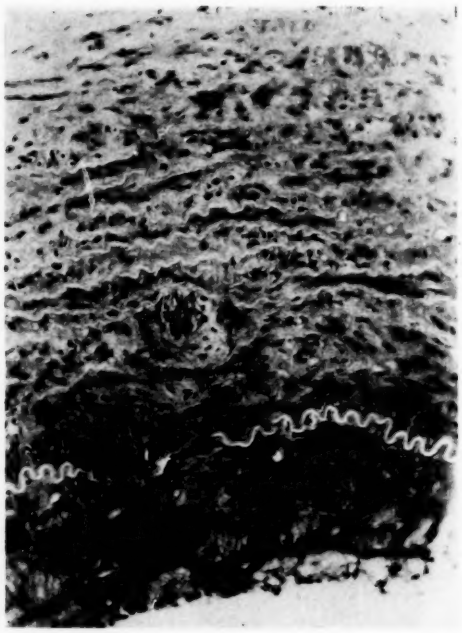

Fig. 5. Cellular proliferation of the thickened intima of an iliac artery; thionine stain. The dark grayish matrix in the intima and in the media is a mucoid material which exhibits metachromatic staining properties. Degeneration of a part of the internal elastic membrane is evident: monkey, 11-month deficiency.

plastic growths on histologic examination. In many cases, the pathologic alterations are quite striking. Again, it is not known at this time how much pyridoxine is needed to prevent this pathologic process in the liver. However, it has been observed in animals receiving 30 to 60 μg of vitamin B_6 daily. The appearance of a normal liver and the characteristic gross alterations may be seen in Figures 6 to 8.

Dental Caries in Pyridoxine Deficiency

Finally, we wish to direct attention to the occurrence of dental caries in pyridoxine deficiency. These data will be reported in detail separately.[13] However, they appear of sufficient interest and potential importance to record at this time.

The great majority of the animals when received in the laboratory weighed from 1800 to

Fig. 6. Normal liver, control monkey. Total time on experimental diet, 4 years, 7 months.

Fig. 7. Enlarged liver with fatty metamorphosis, deficient monkey; complete deficiency, 12 months; partial, 1.5 months.

Fig. 8. Enlarged liver with fatty metamorphosis with some degree of cirrhosis and with regenerating nodules, deficient monkey; complete deficiency. 3 months; partial, 9 months.

2500 grams and were estimated to be from 12 to 18 months of age. On arrival at the laboratory, the animals were usually placed on the synthetic diet with full supplementation. Most of them showed an initial, relatively rapid gain in weight.

In the short-term experiments, little or no effect of pyridoxine deficiency was manifest in the teeth of the animals. However, in those which had been maintained in the deficient state for periods of two years or longer, the frequent occurrence of dental caries became evident. These animals were three or more years of age, and the second dentition had begun or was complete. The degree of dental caries encountered in some of the pyridoxine-deficient animals was quite striking. When this became evident we began a more systematic study of the teeth in our experimental animals, and it became a part of the routine examination to preserve the jaws for a careful evaluation of the extent of caries. The observations will be briefly summarized later in this report. Only the animals that had been on the experimental diet for two years or longer have been considered. In some instances we had preserved only one jaw.

Our observations may be briefly summarized as follows: In eight control animals, 213 teeth were available for study. Caries occurred in 34 teeth, an incidence of 16 per cent.* In five animals subjected to equally prolonged deficiency of vitamins other than pyridoxine, 130 teeth were available for study. Twelve were carious, an incidence of 9 per cent. In seven animals subjected to protracted pyridoxine deficiency, 149 teeth were available for study; 83, or 54 per cent, of the teeth were carious. It should be recalled that the second teeth were in a developmental stage in the early part of the experiment. It is likely enough that defects in the development incident to pyridoxine deficiency rendered the teeth particularly vulnerable to caries after they had erupted.

* In only one animal of this series were the caries prominent. This animal had been maintained on the synthetic diet for $6^1/_2$ years. The diet contained alpha protein, while casein was the protein source in most other animals.

DISCUSSION

It is not known whether man suffers from vitamin B_6 deficiency. It would, however, be surprising if this were not so. The important role of pyridoxine in metabolism (particularly of proteins) is being increasingly recognized. While deficiencies of most water-soluble vitamins result in fairly characteristic external manifestations, pyridoxine deficiency is subtle in its development, exhibiting few if any distinctive clinical features. Furthermore, our studies have shown that what may be called partial pyridoxine deficiency resulting from suboptimal intake over extended periods of time also produces degenerative changes in the arteries, hepatic lesions, and dental caries. May not similar mechanisms operate in man? Actually, little is known of the pyridoxine intake needed to maintain an optimal metabolic state in man. It has been estimated that the average daily intake of pyridoxine by man is approximately 1.5 mg.[14] If this is the average, many must fall below it. There is reasonable doubt whether this amount is adequate for optimal metabolism. In a recent analysis of the problem, Schroeder[15] arrived at the same opinion. Our observations previously noted in this report indicate that the monkey requires something more than 50 micrograms per kilogram per day for optimal growth. If this ratio is applied in man, the requirement for an individual of 70 kilograms would be 3.5 mg, or about $2^1/_2$ times the average daily intake. Our studies in man and in the monkey indicate that the storage or tissue reserves of pyridoxine are limited. Very short periods of complete deprivation of pyridoxine result in a measurable defect in tryptophan metabolism.[16]

During the past several years we have done large numbers of assays of the transaminase activity of whole blood in man.[17] Rarely have we found maximal values of transaminase activity. Administration of pyridoxine in daily doses of 10 to 15 mg for 4 to 6 weeks will regularly cause a 30 to 100 per cent increase of the transaminase activity of whole blood. Reference has been made previously to the relationship of transaminase activity of the blood and tissues of the monkey to the intake[9] of pyridoxine.

In a consideration of the pathogenesis of arteriosclerosis, it should be recalled that it is essentially a universal disease in the American populace. Variations encountered are those of degree and rapidity of development. Based upon studies of averages, the extent of the disease process increases with each decade.[18,19] In any study, then, it is not possible to clearly separate groups of individuals with and without arteriosclerosis. The only clinical separations possible are cases with manifest evidence of the disease (e.g., coronary occlusion with infarction, angina pectoris, occlusive cerebrovascular accidents, and intermittent claudication) and those without manifest evidence of the disease. In fact, the extent of the disease process may be marked in some individuals without clinical manifestation.

The view that pyridoxine deficiency may be a factor in the pathogenesis of arteriosclerosis is based upon the essential morphologic similarity of the experimentally produced lesions and those occurring spontaneously in man. This view does not preclude operation of other factors, such as excess fat and cholesterol intake. There is evidence that pyridoxine is involved in fat metabolism. The subject has recently been reviewed by Schroeder.[15] We presented evidence that, if the pyridoxine-deficient animal is fed added cholesterol (1 per cent cholesterol added to the basal diet), the level of this steroid in the blood rises higher than it does in control animals even though the latter consume significantly greater amounts of cholesterol because they eat considerably more.[20]

At the present time, there is no direct evidence that pyridoxine deficiency is a contributory factor in the pathogenesis of arteriosclerosis, dental caries, or hepatic cirrhosis. However, the experimental data presented strongly indicate that this may be so. There is no precise knowledge of the pyridoxine requirement in man. Arteriosclerosis and dental caries are essentially chronic and universal diseases evolving over periods of years. It may be that these diseases represent the toll of a smoldering, disordered metabolism resulting in part from years of pyridoxine deficiency.

SUMMARY

During the past six years, extensive studies of pyridoxine (vitamin E_6) deficiency in the rhesus monkey (*Macaca mulatta*) have revealed pathologic alterations which suggest that deficiency of pyridoxine may be of importance in the pathogenesis of human disease. Some 40 animals have been studied. The animals were maintained on an essentially synthetic diet fed in tablet form containing 73 per cent sucrose, 18 per cent vitamin-free casein, 2 per cent corn oil, and the essential vitamins and minerals.

Animals subjected to pyridoxine deficiency regularly develop alterations in blood vessels which bear a very close similarity to arteriosclerosis as it occurs spontaneously in man. The experimental lesions are closely analogous in character and distribution to those found in man. In the experimental animals, the vascular lesions develop after five to six months of complete deprivation of this essential nutrient.

Other pathologic changes which frequently occur are fatty metamorphosis and cirrhosis of the liver. Comparable lesions have not been found in control animals or in those subjected to other nutritional deficiencies. In animals maintained for two years or longer on the synthetic diet and given inadequate supplements of vitamin B_6, the incidence of dental caries in the second dentition is unusually high.

Attention is called to the essentiality of vitamin B_6 in metabolism, particularly of proteins. It is problematic whether or not the average daily intake of pyridoxine in man (1.5 mg) is adequate to meet the metabolic needs.

The question arises whether or not long-term suboptimal intake of pyridoxine may be a contributory factor in the pathogenesis of the important human diseases—arteriosclerosis, dental caries, and cirrhosis of the liver. It is hoped that future investigations will supply an early answer to this important question.

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DISCUSSION

DR. GLADYS EMERSON (Rahway, N. J.): We have been very much interested in the whole problem of vitamin B_6 deficiency. As Dr. György indicated, the Merck laboratories have been working in the field of vitamin E_6 since 1934. Indeed, vitamin B_6 has come to life. We have been concerned with the different responses of varying species to E_6-avitaminosis. Dr. György has mentioned that there are several signs of the deficiency state. One of these is the acrodynia that he described. This is seen in rats; however, it does not occur in the monkeys maintained on a vitamin B_6-deficient diet, nor is it observed in the dog. The microcytic anemia first described by Helmer, Jukes, Fouts, and Lepkovsky is seen in the dog, but not with regularity in the monkey. The tryptophan load test is applicable to practically all species. Dr. Vilter, perhaps, will say a bit more about this. Dr. McHenry will tell us about his alanine load test that runs more or less parallel. There are other manifestations of a deficiency in vitamin E_6. The one that is foremost in rats and monkeys is a loss of weight. Dr. György has pointed out that the weight losses observed in rats could not be considered specific. In the monkey, as indicated by Dr. Rinehart, the weight losses are very great. Our findings confirm and extend his observations. Weight losses were not seen in our dogs. The animals had a dry scaly skin, perhaps similar to the seborrheic type of dermatitis that has been described by Dr. Vilter. Dr. Rinehart, in referring to his diet, stated that it had a high concentration of calories. It should be pointed

out that the ration is low in fat, namely, 2 per cent corn oil. Dr. György has mentioned his early work in the '30's on the sparing action of fat on vitamin B_6. At the present time I am wondering perhaps if vitamin E does not have a sparing effect as well. We have observed certain signs of vitamin E deficiency in our monkeys, such as brown pigmentation occurring in practically all tissues, and a wasting of muscle. Wheat germ oil and corn oil, which were supplied by some investigators, are good sources of vitamin E.

Dr. Day and Dr. Dinning have recently reported a vitamin E-vitamin B_6 interrelationship in the monkey. The question of fat is particularly significant, I believe, not only in that a more severe vitamin B_6 deficiency state develops as a result of feeding a diet low in fat (e.g., tests of Rinehart and Greenberg), but also that this low intake of fat may possibly decrease the severity of the arteriosclerotic lesions. There has been a great deal of interest in the influence of fat on the production of arteriosclerosis in experimental animals and on the incidence of the disease in man.

Growth was poor during a period in which our monkeys were fed a mixture of natural foods consisting of Purina pellets, oranges, bananas, apples, whole wheat bread, grapes, and occasional peanuts. (A recent experiment has demonstrated that even the addition of milk and eggs does not promote better growth.) However, when the animals were transferred to the diet of Rinehart and Greenberg plus 1 mg of pyridoxine daily, the slope of the curve was greater than during the previous period when natural foods were given. After five weeks of this treatment, pyridoxine was withdrawn from half of the animals. The animals began to lose weight within two weeks following the omission of vitamin B_6 from the diet. This would indicate either that vitamin B_6 is stored to only a limited extent by the monkey or that 1 mg of pyridoxine per day provided a marginal intake of vitamin B_6 for monkeys subjected to the treatment described. It is surprising to learn from Dr. Rinehart that the vitamin requirement of the rhesus monkey is 50 μg per kg of body weight per day.

Dr. Boxer has determined the pyridoxal phosphate in whole blood of a number of domestic and laboratory species fed natural food diets. He has found that the values ranged from 25–100 millimicrograms per ml. In man and in monkeys more than 80 per cent have values below 10 millimicrograms per ml. When the monkeys were transferred to the purified diet with vitamin B_6, the pyridoxal phosphate fell within the range for other species. When vitamin B_6 was withdrawn, the pyridoxal phosphate level declined slowly.

DR. CHARLES W. MUSHETT (Rahway, N. J.): I have been reminded that time is short and so I am going to discuss as quickly as possible some of the pathologic findings in pyridoxine-deficient monkeys and dogs from a project on which Dr. Emerson and I have been collaborating. Initially, I should like to show you some of the arteriosclerotic lesions of our vitamin B_6-deficient monkeys which I believe you will agree are very similar to those shown earlier by Dr. Rinehart. Also, I should like to show you what we believe to be the first observation of arteriosclerosis in the pyridoxine-deficient dog.

In all pyridoxine-deficient monkeys examined to date we have observed arteriosclerosis in the lower abdominal aorta and also in the common iliac arteries. The plaques occur as pale, longitudinal raised areas. At times an abdominal plaque is seen to continue into the iliac artery. The monkeys under discussion had received the vitamin B_6-deficient diet of Rinehart for periods varying from six to ten months. Microscopic examination of the abdominal aorta of control monkeys reveals the intima to be a relatively thin layer above the conspicuous internal elastic membrane. In marked contrast, a section from a comparable segment of the abdominal aorta of a vitamin B_6-deficient monkey shows the intima to be markedly thickened, frequently up to ten times or more that of the normal. Increased numbers of fibroblasts are present and there is a large amount of pale staining, mucinous-like ground substance. A variable increase in ground substance may also be present in the media, which, as a result, appears less dense than normal. In testicular arteries the intima shows marked

thickening to the extent that it may be even wider than the medial layer. The intima is thickened rather uniformly around the lumen and shows an increase in ground substance and fibroblasts. The intima of the control testicular artery, in contrast, is extremely thin and has but little connective tissue. Although the testes of both the deficient and control monkeys in this series were immature and showed no spermatogenesis, it is of interest to note that the seminiferous tubules of the deficient animals' testes are more hypoplastic than those of the controls.

In the kidneys of deficient monkeys, the large- and medium-sized arteries were found to be arteriosclerotic. Sections show the intimal fibrosis to encircle the lumen or to appear as multiple raised plaques. The internal elastic membrane exhibits splitting and duplication. Arteriosclerotic lesions have also been observed in vitamin B_6-deficient monkeys in the following organs: heart, lungs, liver, adrenals, pancreas, ovary, uterus, thymus, colon, and femoral bone marrow. Examinations to date have not revealed the presence of lipid in any of these lesions.

A comparison of organ weight:body weight ratios shows that many of the organs, including the liver, kidneys, heart, adrenals, thyroid, and pituitary, are relatively enlarged in the pyridoxine-deficient monkeys. The mean relative adrenal gland weight of the deficient animals is about seven times that of the controls. Of particular interest is the fact that these glands show an absolute as well as a relative increase in size. The adrenal is not, however, increased uniformly in the various zones. The fasciculate and reticular zones are increased in width, but the zona glomerulosa is narrower at times than normal and shows less lipid. In the liver of the deficient monkey we have seen the fatty change pointed out by Dr. Rinehart. Our control monkeys show this also, but to a lesser degree. In contrast to the striking deposition of iron pigment in the livers of pyridoxine-deficient dogs, little or no iron is demonstrable in the livers of the deficient monkeys.

Dogs fed for protracted periods a diet adequate in all dietary essentials except pyridoxine have also shown arteriosclerotic plaques. These are grossly discernible and occur in the lower abdominal aorta, as in the monkey, and in the ascending aorta as well. Microscopically the lesions are similar to those just described for the monkey. We believe the present report constitutes the first observation of arteriosclerosis in the pyridoxine-deficient dog. One of the dogs examined showed rough, gritty areas in the ascending aorta which were elevated a millimeter or more. In sections these were noted to show striking calcification in the media. We have not, until recently, seen calcification in the arteries of deficient monkeys, although I believe Dr. Rinehart noted this earlier. One of our monkeys receiving the Rinehart diet plus 2 per cent cholesterol showed calcification in the media of arteries in femoral bone marrow.

In addition to dental caries in pyridoxine-deficient monkeys, which Dr. Rinehart has also seen, we have noticed something hitherto undescribed, namely malalignment of the teeth. Whether the malalignment has a similar basis to that of arteriosclerosis I do not know, but one might speculate that the ground substance of connective tissue is also involved here. Further study, of course, is required to determine this. The malalignment consists principally of improper angulation and spacing of the teeth. Two examples (as shown by photographs) will illustrate this. In the first example, the central incisors of the mandible are inclined backward and because of this have cut into the gingiva of the upper jaw producing hemorrhage and necrosis. Just anterior to the maxillary canine teeth are indentations in the gingiva due to the lower canine teeth. In the second example, relatively deep indentations in the gingiva of the mandible are seen to be the result of impingement by the upper canine teeth. The latter, instead of slanting slightly forward as is normal, are inclined straight downward. The left mandibular canine tooth is angulated almost straight up instead of being inclined slightly forward. This malalignment resulted in the gingival indentation just forward of the left maxillary canine tooth.

DR. NORMAN OLSON (Nashville, Tenn.): We have studied the albino rat exclusively and

have found no evidence whatsoever of atherosclerosis, arterial or arteriolar disease. We have found a consistently elevated systolic blood pressure in our vitamin B_6-deficient animals. This symptom is, of course, a part of the syndrome complex indicating damage to the cardiovascular system. Atherosclerosis may occur in the vitamin B_6-deficient rat at later times, although our experiments were carried on for approximately 34 weeks. This is considerably longer than the experiments on monkeys and dogs when the life span is taken into account. We found no hematologic changes in the vitamin B_6-deficient albino rat. This agrees with the work of Agnew who found no changes in the albino rat but degenerative changes in the hooded rat. Our original studies were done exclusively on males and further unpublished studies on females have shown the same picture. I think that this is the first well-documented production of hypertension in an animal due to a specific vitamin deficiency.

Neurochemical Aspects of Pyridoxine Metabolism and Function

By DONALD B. TOWER, M.D., PH.D.*

IMPORTANT functions in neuronal metabolism have been established for most of the vitamins of the B-complex group. Investigation of the neurological signs and brain metabolism of thiamine-deficient pigeons was responsible for the elucidation of the role of thiamine, as diphosphothiamine (cocarboxylase), in pyruvate metabolism. Nicotinamide (niacin) has been shown to be the active portion of the pyridine nucleotides and riboflavin similarly for the flavoproteins. The importance of energy metabolism to the neuron has established these three vitamins as essential to proper neuronal function. The discovery of coenzyme A with its pantothenic acid component identified the final link in the utilization of the acetyl fragment from pyruvate. This has a special significance for nervous tissue, since acetyl-coenzyme A subserves three important functions therein: synthesis of acetylcholine, an important peripheral and probable central transmitter; formation of citrate, the first step in the Krebs cycle for production of cellular energy; and synthesis of the complex and prominent cerebral lipids. Recently folic acid and vitamin B_{12} have been added to the list of B-complex vitamins essential to neuronal function. It is not surprising that deficiencies of these vitamins result in striking neuronal dysfunctions. In each case myelinated structures, both central and peripheral, are primarily involved. Only thiamine deficiency, in certain species at least, results, in addition, in hyperactivity phenomena, such as the opisthotonic seizures of pigeons.

Pyridoxine (vitamin B_6) must now be added to the list. Neuropathies, myelin degeneration and the like also occur in animals and in man associated with pyridoxine deficiency. In contrast to deficiencies of all the other B-complex vitamins, pyridoxine deficiency is also associated with a striking species incidence of convulsions. These data are summarized in Table I. These observations establish the importance of pyridoxine for normal neuronal function and activity. Studies of the metabolic role of pyridoxine further support this conclusion. Pyridoxine appears to be unique among vitamins of the B-complex group in another way, since excessive amounts are toxic for the central nervous system, resulting in signs and pathologic findings resembling those seen in deficiencies of the vitamins (Table II). With the recent demonstration that pyridoxine is essential for human nervous system function it is important to review the neurochemistry of pyridoxine.

From the Section of Clinical Neurochemistry, National Institute of Neurological Diseases and Blindness, Bethesda, Md.

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PYRIDOXINE AND NEURONAL METABOLISM

Function and activity of nervous tissue depend almost exclusively upon energy derived from oxidative metabolism of glucose. Two basic concepts stem from this fact. When energy supplies fail, either from lack of oxygen or deprivation of glucose, dysfunction and irreparable structural damage rapidly ensue. Because of the blood-brain barrier mechanism, together with the dependence upon glucose, many components of central nervous tissue are synthesized *in situ* from carbohydrate metabolites. The heart of the system concerned with both these aspects of neuronal metabolism is the Krebs tricarboxylic or citric acid cycle, illustrated schematically in Figure 1. It is here that metabolic interchanges occur, which, in the central nervous system, permit glucose to

be utilized for the production of cellular energy, as high-energy phosphate compounds like adenosine triphosphate (ATP), and for the synthesis of fatty acids and amino acids to build lipids and proteins. Krebs[80] has emphasized that these metabolic reactions represent the final common pathway for utilization of practically all foodstuffs by the body. In the nervous system, where dependence upon glucose is very high, this set of reactions is of fundamental importance to the economy of the neuron. Disturbance of function at one point may affect numerous other functions which are mutually interdependent. So many of the interchange reactions are catalyzed by coenzymes derived from B-complex vitamins (as illustrated in Figure 1) that neural dysfunctions associated with deficiency of one or more of these vitamins are understandable.

TABLE I

Species Incidence of Neurologic Symptoms Associated with Pyridoxine Deficiency

Species	Convulsions			Peripheral neuropathy and/or spinal cord dysfunction			References
	No B_6*	Desoxy B_6*	INAH*	No B_6	Desoxy B_6	INAH	
Chicken	+	−	−	(+)	−	−	78, 84
Duck	+	−	−	+	−	−	67
Turkey	+	−	−	(+)	−	−	19
Mouse	−	+	+	+	−	−	22, 64, 73, 102, 108, 124
Rat	+	−	+	0	0	−	31, 37, 38, 40, 61, 85, 100, 103
Guinea pig	+†	−	+	−	−	−	40
Cat	−	+	+	−	−	−	40, 64
Dog	+	+	+	+	−	−	6, 40, 47, 137
Pig	+	−	−	+	−	−	32, 46, 71, 83, 138, 161, 162, 163
Monkey	(+)	+	+	−	−	−	40, 41a, 64
Beef	+	+	−	+	−	−	77
Man	+	+	+	(+)	+	+	14, 16, 17, 36, 52, 70, 72, 73, 98, 108, 130, 133, 134, 156

* No B_6 = diet induced deficiency; Desoxy B_6 = desoxypyridoxine; INAH = isonicotinic acid hydrazide.
+ = observed; (+) = observation or significance in doubt; − = no data; 0 = not observed.
† Reid, M. E.: Unpublished data.

TABLE II

Toxicity of Pyridoxine and Related Compounds for the Nervous System

Mouse	Convulsions—death	References
	LD_{50} mg/kg i.p.*	
Thiosemicarbazide	15	102
Semicarbazide	100	73
Isonicotinic acid hydrazide	150	64
4-Desoxypyridoxine	250	64
Toxopyrimidine	500	90
Pyridoxal	500	64
Pyridoxamine	3000	64
Pyridoxine	3000	64
4-Pyridoxic acid	3000	64

Compound	Convulsant dose	CNS degeneration dose	References
	mg/kg	*mg/kg*	
Rat: Pyridoxine-HCl	3700	3000	3, 154
Dog: Pyridoxine-HCl	—	2500	3
Man:			
Isonicotinic acid hydrazide	35–40	16–24	17, 108
Desoxypyridoxine	(25)	(10)	52, 156
Semicarbazide	40	—	134

* i.p.—intraperitoneally.

Lipid Metabolism

The neural lipids comprise 40 to 65 per cent of the total solids of nervous tissue[75] and are important components of the structure of enzyme complexes, cell cytoplasm and membranes, and the myelin sheaths. Most of the neural lipids exist as complexes with protein.[45] They are probably synthesized *in situ* and are in a dynamic state of breakdown, repair, and synthesis in the living animal.[117,132] The mechanisms for these processes reside in the metabolic area just discussed. The neural lipids fall into three main groups: the phospholipids, the glycolipids (cerebrosides), and the sterols. The phospholipids appear to con-

tain more unsaturated fatty acids, especially oleic and arachidonic, than the glycolipids, but the data are very incomplete.[117] The myelin lipids are composed primarily of glycolipids, sphingomyelin, and cholesterol,[74,75] but the factors concerned with their synthesis and breakdown, particularly in demyelination, are poorly understood.[118]

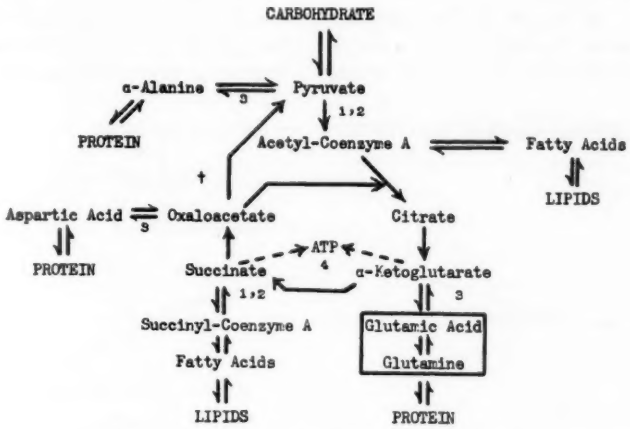

Fig. 1. Intermediary metabolism interchanges. ① Diphosphothiamine (Thiamine). ② Coenzyme A (Pantothenic acid). ③ Pyridoxal phosphate (Pyridoxine). ④ Pyridine nucleotides (Nicotinamide); Flavoproteins (Riboflavin). * Biotin. (Reference: 80.)

Early in the studies of pyridoxine deficiency in animals a relationship between pyridoxine and fatty acid metabolism was recognized.[18] The precise nature of this relationship is still not clear. In pyridoxine-deficient rats total body fat is decreased to about 10 per cent of the content in pair-fed control animals receiving supplemental pyridoxine, whereas the protein content of the two groups is essentially the same.[7] Normally the rat converts 30 per cent of ingested carbohydrate to fat.[26] In the pyridoxine-deficient rat there is no impairment in absorption of carbohydrate, fat, or protein in the diet, and supplemental fat in the diet will restore body fat to levels comparable to control animals.[26] The fatty acids present in the deficient rat are more unsaturated, with a relatively greater percentage of arachidonic acid, but the levels of phospholipids and sterols are maintained at control values.[26,39,123] Acrodynia, the dermatitis typical of pyridoxine deficiency in the rat, responds not only to pyridoxine administration but also to therapy with linoleic acid.[18,164] Linoleic acid is also of some value in correcting depletion of body fat.[39] In fat deficiency in the rat linoleic acid or arachidonic acid are effective in treatment. Linoleic acid is probably the precursor of arachidonic acid in the rat, and dietary deficiencies of these acids are accentuated by pyridoxine deficiency.[59,147,148]

These and other observations have led Sherman[123] to conclude that in the pyridoxine-deficient rat there is a sparing of the breakdown of unsaturated fatty acids with preferential metabolism of the more highly saturated fatty acids. Beaton *et al.*[7] believe that pyridoxine deficiency affects the rat's "energy production," depriving it of surplus food for storage (as fat), but Fried and Lardy[49] suggest that the findings may be a result of impaired amino acid and protein metabolism which deprives the rat of readily available reserves for fat and carbohydrate synthesis. In either case, pyridoxine appears to be concerned in numerous phases of lipid metabolism, including oxidation, synthesis (especially from protein), deposition, and possibly transport with the unsaturated fatty acids, particularly linoleic and arachidonic acids, being intimately involved in this relationship.[123]

Most of the foregoing observations and con-

clusions are based upon studies in the rat. The rat does not exhibit nervous system signs (Table I) or pathologic changes[61] which can be related to impaired metabolism of neural lipids. Neuropathies or myelopathies are conspicuously absent from the many studies on pyridoxine-deficient rats. In other species, notably the pig[46,138,162,163] and the dog,[137] frank

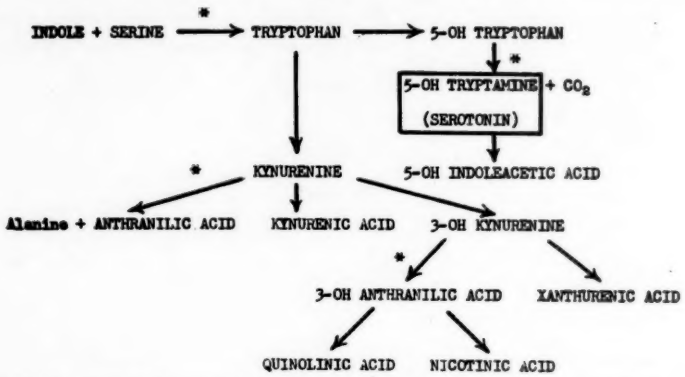

Fig. 2. Tryptophan metabolism. * Requires pyridoxal phosphate. (References: 150, 151.)

myelin degeneration and associated clinical signs have been observed for both peripheral nerve and spinal cord (Table I). The problem is of more than academic interest because of the incidence of dysfunction of myelinated systems in a number of species, including man, and because of the structural and functional importance of neural lipids to the nervous system. No biochemical studies of neural lipids in pyridoxine deficiency have been reported. Such studies would be important to the possible role of pyridoxine in neural lipid metabolism and to mechanisms underlying degeneration of myelin structures.

Tryptophan Metabolism

Lepkovsky and associates[86] first observed that pyridoxine-deficient rats excrete xanthurenic acid, that its excretion increases after a tryptophan load, and that it disappears with restriction of tryptophan intake or on pyridoxine supplementation. Since then considerable attention has been focused on the relationships between pyridoxine and tryptophan metabolism. Greenberg and co-workers[58] found xanthurenic excretion following a tryptophan load to be one of the earliest signs of pyridoxine deficiency, a finding present in mice, rats, pigs, dogs, monkeys, and man during pyridoxine deficiency. In man the so-called pyridoxine antagonists, desoxypyridoxine[54,156] and isonicotinic acid hydrazide[16,17] when administered also result in xanthurenic acid excretion in response to a tryptophan load. The presumed scheme of tryptophan metabolism with the pyridoxine-dependent steps are outlined in Figure 2.[151]

A specifically important role for tryptophan in neuronal metabolism has only recently been demonstrated. Udenfriend and Titus[150] have shown that tryptophan is converted to 5-hydroxytryptophan by mammalian tissues and decarboxylated to 5-hydroxytryptamine or serotonin. Serotonin is present in significant concentrations in the central nervous system[2,104] and its antagonism by lysergic acid diethylamide (LSD) has led Gaddum[50] and Woolley and Shaw[165] to suggest an essential role for serotonin in neuronal function. Erodie and co-workers have recently demonstrated that serotonin has a reserpine-like action in sedating mice[125] and have presented evidence for the mediation of reserpine action by its mobilization of brain serotonin.[104] The decarboxylase converting 5-hydroxytryptophan to serotonin is highly specific for this reaction[34] and is present in brain in high concentrations, apparently paralleling the concentrations of serotonin.[149] In view of the effects of lysergic acid diethylamide on mental function[136] and its

blockage of sensory synapses,[43] the "tranquilizing" action of reserpine,[120,146] and the relationship of serotonin to these compounds,[104] an important role for serotonin in neuronal activity is strongly suggested.

5-Hydroxytryptophan decarboxylase has now been shown to require pyridoxal phosphate as coenzyme.[149] As suggested by preliminary experiments,[34] the coenzyme is bound very tightly to the apoenzyme, with marked resistance to dissociation, and, in addition, the enzyme shows greater affinity for substrate and for carbonyl-trapping agents such as semicarbazide than other amino acid decarboxylases.[149] As might be expected, an effect of pyridoxine deficiency on this enzyme system has been difficult to demonstrate in the mouse or rat.[149] It would seem unlikely that some of the less specific effects of pyridoxine deficiency such as depression, irritability, and apathy are related to dysfunction of this system. However, the neurotoxic effects of the hydrazides deserve reconsideration in the light of these findings.*

Amino Acid and Protein Metabolism

The most important function of pyridoxine and related compounds is in the form of the coenzyme, pyridoxal phosphate, in amino acid and protein metabolism. The reactions for tryptophan represent one example of the many functions of pyridoxine coenzymes. The two principal types of reactions catalyzed by pyridoxal phosphate are decarboxylation and transamination. These are exemplified by the metabolic reactions for cysteine shown in Figure 3.

The studies of Awapara and Wingo,[5] Bergeret *et al.*,[13] Hope,[68] and Chapeville and Fromageot[29] have established that cysteine is first converted to cysteinesulfinic acid (not cysteic acid as previously supposed). Cysteinesulfinic acid can be metabolized by either of two routes: decarboxylation to hypotaurine, which is readily oxidized to taurine, or transamination with a keto-acid (α-ketoglutarate) to form an intermediate, β-sulfinylpyruvic acid, which readily loses its sulfur moiety to become pyruvic acid. The pyruvate formed can transaminate with an amino acid (glutamic acid) to become alanine while reconstituting the keto-acid.[5,30] The two transaminations or the decarboxylation are all catalyzed by pyridoxal phosphate as coenzyme. These reactions are of interest to neurochemistry since taurine has been found in mouse brain[114] and in cat and human cerebral cortex.[143] In the latter two species it is one of the seven most prominent amino compounds. Traces of cysteinesulfinic acid have been found in rat brain together with small amounts of hypotaurine.[12]

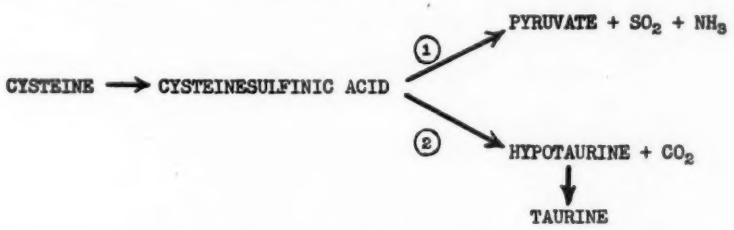

Fig. 3. Cysteine metabolism. ① Transamination with α-ketoglutarate: pyridoxal-phosphate dependent. ② Decarboxylation: pyridoxal phosphate dependent. (References: 5, 12, 29, 30.)

Numerous decarboxylation and transamination reactions, requiring pyridoxal phosphate as coenzyme, have been demonstrated in mammalian tissues. Several excellent reviews have summarized our knowledge on this subject[92a,128,151] and a general mechanism of action for pyridoxal phosphate has been proposed.[96] Only a few of the studies have included brain in

* Now Udenfriend, Weissbach and Bogdanski (*Ann. New York Acad. Sc.*, in press, 1956) have found that, following induction of pyridoxine deficiency in chickens, the serotonin concentration in the brains of such animals is significantly reduced. This finding may be correlated with the impairment in conversion of 5-OH-tryptophan to serotonin (Fig. 2) which they have observed in such brains.

the tissues investigated, but these indicate that brain tissue is very active.[35,113] One of the most important systems for tissues in general and for central nervous tissue in particular is that concerned with glutamic acid. These are summarized schematically in Figure 4. Glutamic and aspartic acids, and to a lesser extent alanine with their respective keto-acids,

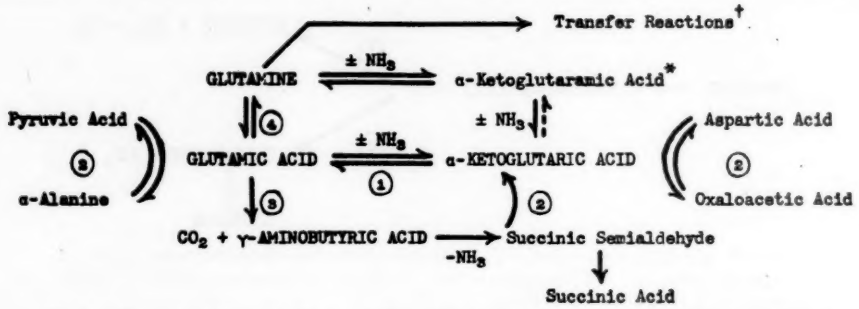

Fig. 4. Glutamic acid metabolism. ① Deamination-amination: DPN dependent. ② Transamination: pyridoxal phosphate dependent. ③ Decarboxylation: pyridoxal phosphate dependent. ④ Amidation-glutaminase: ATP dependent. * Transamination-deamidation (? in brain; ? dependency). † Glutamotransferase (? dependency). (References: 4, 15, 35, 79, 81, 92, 95, 110, 111, 112, 113, 121, 131, 158.)

subserve the majority of transamination reactions. Through them ammonia can be utilized by tissue cells to synthesize their particular amino acid requirements. In addition, the amides, glutamine and asparagine, increase the versatility of this general mechanism by transamination of the amide groups[92,95] or transfer of the glutamyl or aspartyl groups.[121] These reactions are intimately concerned with protein synthesis and with amino acid-carbohydrate interchanges.

The metabolism of glutamic acid and related compounds is particularly important in central nervous tissue. Some 60 per cent of the nonprotein nitrogen component of brain tissue are α-amino compounds and 80 per cent of the latter are glutamic and aspartic acids and related compounds.[159] In all species studied brain contains much higher concentrations of glutamic acid and glutamine than liver or kidney.[157] Glutamic and aspartic acids are probably largely synthesized *in situ* from the appropriate keto-acids of the Krebs cycle, derived from carbohydrate metabolism.[157] Brain also contains a special system for decarboxylation of glutamic acid to γ-aminobutyric acid.[4,111–115,160] This system is not present in other tissues.[113,115] Subsequent studies have demonstrated that γ-aminobutyrate is metabolized by brain via a transamination reaction to succinate.[15,110] This system is present in the brains of all species studied, including man, and is catalyzed in both steps by pyridoxal phosphate as coenzyme.[4,113,115] Slices of cat and human cerebral cortex show an appreciable increase in γ-aminobutyrate content during incubation in a glucose-saline-bicarbonate medium.[143] These and other observations provide evidence for the importance of glutamic acid and related compounds in neuronal metabolism. The subject has been reviewed in detail by Waelsch.[157]

The effects of pyridoxine deficiency on phases of amino acid metabolism catalyzed by pyridoxal phosphate have not been well studied in nervous tissue. Available data, compared with those for liver, are summarized in Table III for the rat, which is the only species studied in this regard. Appreciable reductions in glutamic acid decarboxylase and cysteinesulfinic acid decarboxylase activities have been found with less effect on glutamic-aspartic transaminase activity. In the case of the latter two systems, *in vitro* addition of pyridoxal phosphate restored enzyme activity to control levels, and in all three dietary supplementation with pyridoxine had a similar effect. It is of interest that the single study estimating brain pyridoxine content indicates a much higher normal content for brain than for liver, kidney,

or muscle, but a striking drop in the face of pyridoxine deficiency.[129]

PYRIDOXINE DEFICIENCY AND NEURONAL DYSFUNCTION

In considering the relationships and significance of impairment of enzyme activities associated with pyridoxine deficiency (Table III) for impairments of neuronal function as manifest in convulsions, neuropathies, and myelopathies (Table I), there are several fundamental aspects to be considered.

As a general rule, enzyme concentrations are not the rate-limiting factors for reactions. Most enzymes exist in tissues in excess of apparent requirements. Thus, an appreciable reduction in enzymatic activity, associated with a deficiency state, as measured by usual *in vitro* techniques, may bear little relationship to clinical signs. In thiamine deficiency, for example, cocarboxylase activity of pigeon brain can be reduced to 40 per cent of normal without detectable clinical manifestations. When acute opisthotonic attacks appear, brain cocarboxylase is only about 15 per cent of normal.[99] Caution is, therefore, advisable in attempting to correlate levels of enzyme activity with system dysfunction.

Where data are not available for the tissue in question, findings in other species or on other tissues in the same species cannot be resorted to with impunity. Studies on cysteinesulfinic acid decarboxylases are a case in point (Table III). Rat liver normally shows five times the activity of brain. In the pyridoxine deficiency studies, pair-fed animals receiving supplemental pyridoxine have much lower activities in both tissues, but liver apoenzyme is saturated with coenzyme, whereas brain apoenzyme is only 10 per cent saturated. The saturated levels of activity are the same, indicating appreciable reduction in liver apoenzyme on the restricted diet. In deficient animals neither tissue shows decarboxylase activity, but upon *in vitro* addition of pyridoxal phosphate the brain enzyme activity reached levels comparable to those of pair-fed controls, while liver showed no activity, indicating loss of apoenzyme as well as coenzyme. Even after 45 days on supplemental pyridoxine in the diet, only 40 per cent of the liver activity in pair-fed controls can be demonstrated.[13] These findings for liver have been independently confirmed.[65] It has been pointed out that in the rat liver cysteinesulfinic acid decarboxylase disappears by 14 days of deficient diet, while pyridoxal phosphate is still present in the liver. The sequence of changes in rat liver for this enzyme system is: (a) disappearance of coenzyme bound to apoenzyme (pyridoxal phos-

TABLE III

Brain Enzyme Activities Associated with Pyridoxine Deficiency in the Rat

Diet →	Normal	B_6-def. + B_6*		B_6-deficient		
In vitro addition →	None	None	+B_6al-P†	None	+B_6al-P†	References
A. Glutamic acid decarboxylase:						
Brain (Init./max. rate)	0.77	0.76	—	0.38	0.84‡	116
Brain (Qco_2)	1.5	—	—	—	—	13
Liver (")	0.0	—	—	—	—	13
B. Cysteinesulfinic acid decarboxylase:						
Brain (Qco_2)	1.0	0.18	1.8	0.0	2.3	13
Liver (")	5.4	1.4	1.5	0.0	0.0	13
C. Glutamic—Aspartic transaminase:					0.6‡	
Brain (% transam.)	59.8	58.6	—	41.3	§	119
Liver (")	58.9	62.4	—	42.7	§	119
D. Pyridoxine content:						
Brain ($\mu g/g$)	—	3.01	—	0.33	—	129
Liver (")	—	0.84	—	0.27	—	129

* Pair-fed animals.

† Pyridoxal phosphate.

‡ After addition of dietary vitamin B_6.

§ Corrected by dietary vitamin B_6 or pyridoxal phosphate in another study.[1]

phate will still activate the system at this early stage); (b) disappearance of the apoenzyme protein; and (c) finally disappearance of coenzyme.[68]

Coincident with these changes associated with pyridoxine deficiency, the excretion of taurine in the urine ceases,[13,20] and reappears when pyridoxine is restored to the diet.[20] However, rat liver is still able to metabolize cysteinesulfinic acid *in vitro* and *in vivo* by way of the transamination pathway (Fig. 3), indicating that this system is less sensitive to

TABLE IV

Sensitivity of Pyridoxal Phosphate-Dependent and Related Systems in the Rat to Pyridoxine Deficiency

System	Per cent of control		References
	Liver	Brain	
Decarboxylases:			
Glutamic acid	—	50	116
Cysteinesulfinic acid	0	0	13
5-Hydroxytryptophan	100	—	149
Transaminases:			
Glutamic-aspartic	77, 68	70	25, 119
Glutamic-pyruvic	36, 37	—	25, 93
Cysteinesulfinic acid	13	—	93
Glutamine	100	—	93
Other systems:			
Glutamic dehydrogenase	100	—	8
Urea production	128	—	9
Pyridoxine content	32	10.5	129
Pyridoxal-P content	15–30	—	11
Xanthurenic acid excretion:			
No load	2.5 × Control		
Tryptophan load	12.5 × Control		100

pyridoxine deficiency than the decarboxylase system.[30] The latter system appears to be present in the livers of the rat, guinea pig, rabbit, and dog, but not in the cat.[68] Since the cat produces taurine, some other mechanism or tissue may be involved.

Pyridoxal phosphate functions as a coenzyme in numerous enzyme systems. The preceding discussion also brings up the factor of differences in sensitivity of various enzyme systems to pyridoxine deficiency. Some of these data are tabulated for the rat in Table IV. They have been taken from a number of sources, so that they are not necessarily comparable in terms of diet, length of deficiency, and the like. However, the order of susceptibility to deficiency would appear to be: cysteinesulfinic acid decarboxylase most susceptible, followed by cysteinesulfinic acid transaminase, glutamic-pyruvic transaminase, glutamic decarboxylase, glutamic-aspartic transaminase, and 5-hydroxytryptophan decarboxylase, in order of decreasing susceptibility to pyridoxine deficiency. Similar data for the mouse,[41] the duck,[24] the hamster,[126] and other tissues of the rat[1,119] have been reported. In the monkey and man, the glutamic-aspartic transaminase activity of blood plasma varies with pyridoxine intake even on adequate intakes.[91] The plasma activity from monkeys receiving no pyridoxine for 10 weeks has been reported to be about 10 to 20 per cent of that for monkeys on 1 mg intake per day.[91] The wide range of susceptibilities of enzyme systems to pyridoxine deficiency complicates the interpretation of clinical dysfunction in terms of impairments of enzymatic function.

There is a further complication in the case of pyridoxine because it functions as coenzyme in systems which are concerned with amino acid metabolism and protein synthesis. In the rat, decreased decarboxylase and transaminase activities (Table IV), increased urea formation and elevated blood urea,[7,9] and changes in glutamine metabolism[10] represent more direct evidence. Synthesis of pyridine nucleotides by rat liver and erythrocytes is impaired by pyridoxine deficiency.[82,88] Under certain conditions, nucleic acid synthesis in mice is interfered with.[28] The anemia common to many species during pyridoxine deficiency appears to be due to impairment of protoporphyrin synthesis for heme production.[27] It has been reported that pyridoxine deficiency is associated with appreciable reductions in riboflavin, nicotinamide, and pantothenic acid contents of rat liver and other tissues.[139] Delayed water diuresis in deficient rats has been linked to lack of production or secretion of a cortisone-like substance, possibly due to corticotropin (ACTH) insufficiency.[62] With possibilities for impaired enzyme syntheses, hormonal insufficiencies, defects in porphyrin metabolism, and reduced enzyme activities, the mechanisms by which pyridoxine deprivation produces clinical dysfunctions can be complex indeed.

The use of so-called pyridoxine antagonists

or antivitamins, particularly in man, has further complicated this problem. 4-Desoxypyridoxine and isonicotinic acid hydrazide (INAH) have been most widely employed for this purpose. Stoerck[135] was one of the first to distinguish between the effects of simple dietary pyridoxine deficiency and effects produced by desoxypyridoxine. Other investigators have reported findings which also suggest that desoxypyridoxine is not a true antivitamin.[9,41] In reviewing this subject, Umbreit[152] points out that there is little question that desoxypyridoxine is antagonized by pyridoxine, but the reverse is not necessarily true. Umbreit and Waddell[153] showed that desoxypyridoxine can be converted enzymatically to desoxypyridoxine phosphate, which can compete with pyridoxal phosphate for the apoenzyme and is effective primarily when pyridoxine intake is restricted. Studies on purified pig muscle apotransaminase confirm these conclusions.[94]

There are several alternative modes of action proposed for isonicotinic acid hydrazide (INAH). Evidence has been presented that INAH competes with pyridoxal phosphate for the enzyme site.[21,87,106,166] Other investigators have suggested that INAH, as a hydrazide, acts as a carbonyl-trapping agent and can combine with pyridoxal as pyridoxal isonicotinyl hydrazone.[16] The protective activity of pyridoxine against the toxicity of semicarbazide, and thiosemicarbazide could be explicable on this basis.[40,73,102] In man, INAH is excreted primarily as an acetylated derivative, but in those cases developing neuropathy much less of the acetylated form was excreted.[71] The recent finding that 5-hydroxytryptophan decarboxylase has a strong affinity for semicarbazide which cannot be antagonized by pyridoxal phosphate[149] is further evidence for this possible mode of action.* Finally, there is clear evidence that INAH can be synthesized by tissues into an analogue of the pyridine nucleotides,[167] which is inert enzymatically and in sufficient concentration might interfere with cellular oxidative metabolism.[55]

Five problems relating to pyridoxine deficiency have been considered: levels of enzyme activity, enzyme activities in various tissues, differences in sensitivity of various enzymes, multi-system effects, and so-called antagonists. These problems are important in terms of mechanisms by which pyridoxine deficiency results in impairment of function. In the nervous system, some aspects of normal metabolism are still poorly understood, so that it is difficult to visualize the mode or modes of action of pyridoxine deficiency. This situation applies to the neuropathies and myelopathies encountered. On the other hand, in the sphere of energy supply, production, and utilization, the nervous system is so dependent upon proper functioning of many interrelated systems that it is difficult to single out any system primarily linked with pyridoxine deficiency in causing dysfunction. This appears to be the case with convulsions encountered in the deficient animal or patient. There are some data on the mechanisms underlying the seizure process in brain which can be examined with these points in mind.

EPILEPTIFORM SEIZURES AND PYRIDOXINE

The species incidence of seizures associated with pyridoxine deficiency has been summarized in Table I. Without exception these have responded to supplements of pyridoxine. No lesions have been found in the brains of animals subject to such fits.[61,161] These characteristics categorize pyridoxine-deficiency seizures as being due to a biochemical lesion. In addition, the twos o-called antagonists, desoxypyridoxine and isonicotinic acid hydrazide and closely related compounds (Tables I and II), are quite general in their convulsant properties.

The recognition that pyridoxine is essential for human nutrition stems from the study of Snyderman and associates,[130] first reported in 1950, on two infants given a pyridoxine-deficient diet. One of these infants developed

* Recently Williams and Abdulian (*J. Pharm. & Exper. Therap.* 116: 62, 1956) have reported that the dog, within 30 to 60 minutes of administration of INAH, excretes pyridoxine compounds in amounts up to ten times controls. The increased excretion is associated as a complex with the hydrazide (presumably pyridoxal isonicotinyl hydrazone).

seizures on the 76th diet day which were corrected by pyridoxine administration. Confirmation of this observation came shortly.

Between 1951 and 1954 a considerable number of infants on a pyridoxine-deficient commercial formula developed seizures which were rapidly controlled by change of formula or supplementation with pyridoxine.[14,36,98] Coursin[36] reported on 54 such babies, demonstrating not only the effectiveness of pyridoxine therapy in controlling their seizures, but also the dramatic improvement in their electroencephalograms within minutes after injection of pyridoxine.

At this time Hunt *et al.*[72] published an account of an infant with seizures which were dependent for control on continued doses of pyridoxine. The authors termed this "pyridoxine dependency" and had followed the case 21 months at the time of reporting. The maternal history is of interest. The case reported involved the third child. In her first pregnancy the mother had had no complications and bore a normal baby. The second and third pregnancies were complicated by hyperemesis gravidarum, treated by pyridoxine. The second child developed neonatal convulsions and died; the third child is still pyridoxine-dependent. The associations may be coincidental, but a correlation of pyridoxine therapy during pregnancy with neonatal seizures requiring pyridoxine for control cannot be ruled out. Unna[154] has reported that seizures induced in rats by a single massive dose of pyridoxine persisted for one to two weeks. Emerson[41a] has observed seizures develop in a monkey, which had been carried for many months on a pyridoxine-deficient diet, after reinstitution of pyridoxine administration in low dosage. Studies on suckling rats and pigs are consistent with the reports on human infants deprived of pyridoxine.[37,83,103] Litters suckled by lactating mothers on pyridoxine-deficient diets soon developed convulsions, often with fatal consequences. Pyridoxine administration to the mother or to the sucklings gave complete protection.

Studies such as these led Fox and Tullidge[48] to a trial therapy of 20 to 100 mg per day of pyridoxine in a group of eight adolescent patients with petit mal or mixed type seizures. Treatment for periods of three to eight weeks were without effect on seizure frequency. In 1951, Ernsting and Ferwerda[42] reported on 14 young patients with petit mal treated with 60 to 120 mg of pyridoxine daily for periods of 10 to 20 months. In five patients the seizures ceased, three experienced a significant decrease in seizure frequency, and six remained unaffected. Attempts to confirm these results have been without significant results,[36a,51] and efforts to demonstrate evidence of pyridoxine deficiency in seizure patients by the tryptophan-load test[58] have also been unsuccessful.[51]

A series of studies on samples excised from epileptogenic foci in human cerebral cortex has demonstrated several significant metabolic abnormalities, which suggest that focal cortical seizures may be due to a biochemical lesion. Cholinesterase activity is elevated,[105,144] production of "bound" acetylcholine is impaired,[144] glutamic acid is abnormally metabolized,[140] and maintenance of intracellular potassium is apparently defective.[141] The possibility that these defects of metabolism in epileptogenic cerebral cortex result from an underlying defect in the production or utilization of cellular energy has been suggested.[141,142] Experimental reproduction and control of these metabolic defects has been achieved. The toxic agent, methionine sulfoximine, which produces seizures in all species tested, is associated with similar defects in metabolism of samples of cerebral cortex from such intoxicated animals.[141,145] Methionine sulfoximine is known to interfere in some manner with methionine metabolism[109] and with glutamine synthesis.[131] The *in vitro* additions of glutamine, asparagine, or adenosine triphosphate (ATP) to such cortical slices reverse the defects in metabolism but do not detectably affect the metabolism of normal slices.[141,142,145] The lack of consistent histopathologic changes,[143] or of significant differences in neuron density,[144] and the reversal of metabolic abnormalities by glutamine, asparagine, or ATP suggest that these abnormalities constitute a true biochemical lesion.

As a result of these observations, attention has been focused on glutamic acid as probably

being important in the seizure process.[141,142] Epileptogenic cerebral cortex, incubated *in vitro*, exhibits a marked reduction in levels of glutamic acid in contrast to non-epileptogenic samples whose level increases.[140] The loss of glutamic acid in the former cannot be accounted for by loss to the incubation medium either as glutamic acid or as glutamine, γ-aminobutyrate, or α-ketoglutarate and represents, therefore, a metabolic loss within the tissue.[141,143] The nature of this abnormal metabolic utilization of glutamic acid is under study. It can be prevented or compensated for by addition *in vitro* of asparagine, ATP, or α-ketoglutarate.[141]

Pyridoxal phosphate functions as coenzyme in many of the metabolic reactions of glutamic acid (Fig. 4). In pyridoxine deficiency seizures are common (Table I), and brain excitability is increased, suggesting that maintenance of transamination is essential for normal brain activity.[38] In addition, evidence for abnormalities in glutamic acid and glutamine metabolism has been reported in pyridoxine-deficient animals.[7,10] In view of these various considerations, the effect of pyridoxal phosphate* has been tested by the methods summarized above. Results of such studies are shown in Table V, compared to those previously reported for asparagine and ATP.[141,142] Glutamic acid metabolism in samples of cerebral cortex from cats in which seizures had been induced by methionine sulfoximine is not affected by addition of pyridoxal phosphate, in contrast to the corrective effects obtained with asparagine or ATP. In human epileptogenic cerebral cortex, however, pyridoxal phosphate addition appears to have a distinct effect. The findings given in Table V are preliminary in nature and require further study before conclusions may be drawn regarding their significance.

As a result of investigations on human epileptogenic cerebral cortex, summarized above, a clinical trial of the efficacy of L-glutamine and L-asparagine in controlling seizures has been undertaken. A preliminary report on early cases in this study has appeared, indicating significant control of clinical seizures in certain cases and improvement in electroencephalographic abnormalities in cases suitable for such assessment.[141] Doses of L-glutamine and L-asparagine are relatively large (2–3 mM/kg). Studies on pyridoxine-deficient subjects have suggested that a protein or amino acid load may intensify the deficiency.[25,53] Some of the patients have shown minimal responses to L-glutamine or L-asparagine. In addition to variations in absorption and utilization of these amides, the possibility of latent or precipitated pyridoxine deficiency has been considered in these patients.

The effects of injected pyridoxine on electroencephalographic activity of seizure patients is important to assess in this regard. Only one report on non-deficient subjects has been found, which indicated that pyridoxine administered intravenously or by local cortical application results in an increase in potential (amplitude) without change in frequency of the electroencephalogram.[56,57] The effect appeared within one minute and persisted for 30 minutes. No other B-complex vitamins exhibited detectable action. Few seizure patients are suitable for this type of study. An example of one such patient with a consistent 3 per second spike and wave discharge on hyperventilation is shown in Figure 5. In this case control electroencephalograms showed abnormal discharge to occupy 80 per cent of the hyperventilation period. Intravenous pyridoxal phosphate (20 to 40 mg), pyridoxine (100 mg), or pyridoxal phosphate plus L-glutamine (20 mg and 2 mM/kg, respectively) but not glutamine alone resulted in significant reductions in the abnormal discharges during hyperventilation, as summarized in Figure 5.* An effect on amplitude, reported by Gozzano *et al.*,[56,57] was not observed.

The results in Table V and Figure 5 suggested the advisability of therapeutic trials of pyridoxine in seizure patients. The patient illustrated

* Pyridoxal phosphate for these and subsequent studies was kindly donated by the Research and Medical Divisions of Merck and Co., Inc., Rahway, N. J. (Drs. G. E. Boxer and N. T. Ritter).

* The co-operation of Dr. C. Ajmone-Marsan and Dr. C. E. Wells in this study is gratefully acknowledged.

in Figure 5 and another patient, both resistant to the usual anticonvulsant regimes and to L-glutamine, have been tried for two and four months, respectively, on oral pyridoxine hydrochloride in divided daily doses totaling 4 mg/kg. No untoward symptoms were encountered, but no changes in seizure frequency or electroencephalographic abnormalities were observed

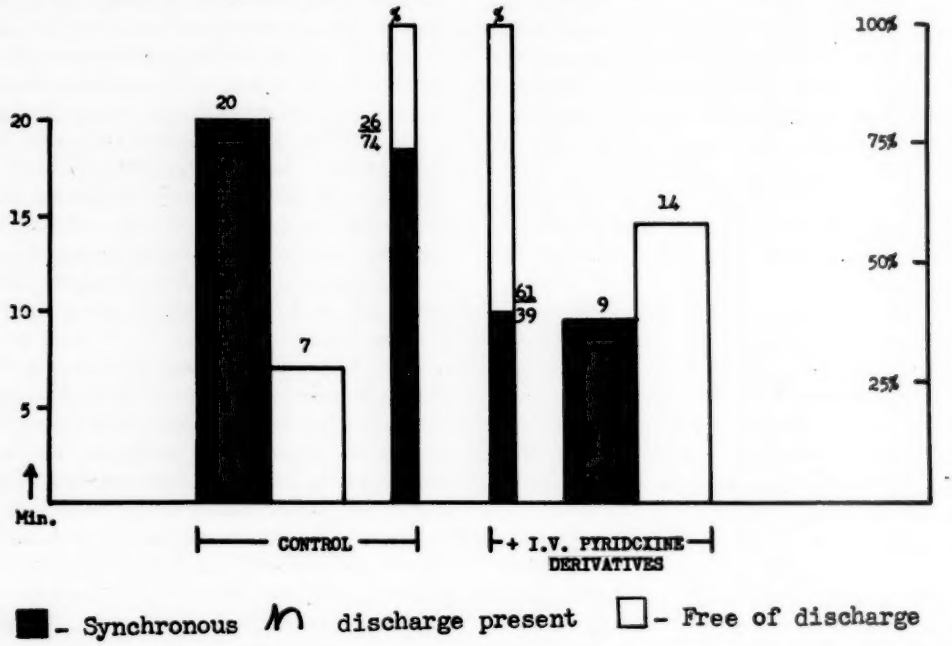

Fig. 5. Electroencephalographic activity during hyperventilation. Patient W. P., male, 19 yrs. wt. 70 kg.

TABLE V

Glutamic Acid Metabolism in Slices of Cerebral Cortex from Normal and Epileptogenic Brain Tissue

Tissue sample	In vitro addition†	Slices incubated 60 min.* Change in glutamic acid	Change in glutamine
		($\mu M/g$)	
Cat cortex:			
Normal	—	+1.7	+2.75
Methionine sulfoximine‡	—	−0.8	+0.85
	10m*M* L-Asparagine	+2.9	0.0
	3m*M* Sodium ATP	+2.5	+0.95
	200 μg Pyridoxal-P§	−0.5	+0.8
Human Cortex:			
Non-epileptogenic	—	+1.4	+1.85
Focal epileptogenic	—	−1.85	+1.9
	10m*M* L-Asparagine	+1.0	+2.4
	3m*M* Sodium ATP	+1.75	+1.65
	200 μg Pyridoxal-P§	+0.2	+4.6

* Changes in incubation medium essentially the same in all runs.

† Additions to normal slices of the three compounds were without detectable effect.

‡ Seizures induced prior to sacrifice with 15 mg/kg methionine sulfoximine intraperitoneally.

§ Per 2 ml incubation medium.

while the patients were on pyridoxine alone or in combination with L-glutamine.

It is clear from these and the previous studies cited that the relationship between pyridoxine and epileptiform seizures is not a simple or clear-cut one except in frank deficiency of the vitamin, and there the mechanisms are not apparent. Further study is needed to clarify the problem. On the basis of the evidence reviewed, it seems likely that a defect in the ability of the neuron to handle amino acids, particularly glutamic acid and glutamine, may be involved. Whether this is the primary difficulty or is secondary to interference with energy production or utilization cannot be decided at present. In addition to infants frankly deprived of pyridoxine, there appear to be other cases requiring pyridoxine supplementation to prevent seizures or other hyperactivity phenomena.[42,44,72] However, this approach is not universally applicable to seizures in man, and there is no simple means at present to select the few which will be benefited by pyridoxine.

PYRIDOXINE METABOLISM

In connection with such studies it is desirable to have information concerning the metabolism of ingested pyridoxine and the relationship between dose and body fluid levels. The latter is important for central nervous system diseases because of the blood-brain barrier mechanism. No published reports of cerebrospinal fluid levels have been found, and data on blood levels and urinary excretion are few indeed.

Vilter[155] has estimated the average human adult intake of pyridoxine to be 2 or 3 mg daily, with 0.8 mg excreted in feces, 3 to 5 mg excreted in the urine (mostly as 4-pyridoxic acid), and 0.2 mg circulating in the blood. Excretion, thus, exceeds dietary intake, at least at ordinary levels of intake, an observation which has been confirmed by a number of investigators.[76,77,89,97,107] Møller[97] found an average increase of 65 per cent in the pyridoxine content of pig fecal suspensions incubated in glucose-saline, and other studies have demonstrated that intestinal flora can synthesize pyridoxine in the cow, sheep, rat, and horse.[89] Absorption of pyridoxine from the intestinal tract of dogs and also that of man is rapid.[122]

In the monkey on control diets supplying 1 mg of pyridoxine daily, levels of total pyridoxine compounds in the blood range from 5 to 21 μg/100 ml (average 11.2 μg/100 ml) in younger animals and from 2.5 to 7.5 μg/100 ml in older animals,[60,91] with about 75 per cent contained in the plasma and 25 per cent in erythrocytes.[60] Total blood levels in man have been reported for 18 normal subjects in the range of 1.7 to 1.8 μg/100 ml, with a rise to about 8.5 μg/100 ml on a pyridoxine intake of 15 mg per day.[91] Other observations indicate that daily doses of 5 to 50 mg of pyridoxine result in blood levels of 5 to 7 μg/100 ml of pyridoxal phosphate.[23] During pyridoxine deficiency in the monkey, total blood levels drop to about 20 to 30 per cent of control values.[60,91]

Most of the excretion of pyridoxine compounds occurs by way of the kidney,[89] with 4-pyridoxic acid[69] accounting for 70 to 90 per cent of the total urinary excretion, regardless of the amount ingested.[76,77,89,97,107] In the dog and in man, the maximum urinary excretion occurs in one to three hours after ingestion, or 30 minutes after intravenous administration of a single dose of pyridoxine, with a recovery of about 20 per cent of the dose in the urine.[122] After oral administration of relatively large doses of pyridoxine compounds (about 1 mg/kg) daily, urinary recoveries for human subjects averaged 38 per cent when pyridoxamine was given, 45 per cent for pyridoxine, and 70 per cent for pyridoxal.[107] The maximum increase in urine output was one hour after ingestion for the fraction as 4-pyridoxic acid and two to five hours for other forms (pyridoxine, pyridoxamine, and pyridoxal), with a return to control levels after 8 and 12 hours, respectively. Only in the case of administration as pyridoxine was there a significant amount (20 per cent) excreted unchanged. A reduction in total urinary excretion to about 30 per cent of control values has been observed in pyridoxine-deficient calves.[77]

In one of the seizure patients previously discussed, who was receiving 4 mg/kg of pyridoxine daily, levels of pyridoxine compounds in the cerebrospinal fluid were determined. The results of these determinations are shown in

Table VI.* Adequate amounts of pyridoxine compounds penetrated into the central nervous system compartment of this patient, with an appreciable fraction in the coenzyme form. From unpublished experiments in cats and rats, 25 to 50 per cent of blood pyridoxal phosphate concentrations are found in cerebrospinal fluid.[23] If these data are applicable to man, the blood levels in this patient would be of the order of 8–16 μg/100 ml pyridoxal phosphate. The cerebrospinal fluid sample was drawn two and a half hours after an oral dose of 50 mg of pyridoxine hydrochloride, with the last previous dose about 10 hours before.

TABLE VI

Cerebrospinal Fluid Levels of Pyridoxal Phosphate and Related Compounds in a Patient Receiving Daily Pyridoxine Hydrochloride

	μg/100 ml*
Pyridoxal phosphate	4.0
Pyridoxal	3.0
Pyridoxine	13.0
Pyridoxamine	None

* Sample drawn 2.5 hrs. after 50 mg pyridoxine-HCl orally. Daily dose 300 mg (50 mg × 6) for 6 days. Patient: male, 19 yr., wt. 70 kg.

From the observations it is apparent that absorption of an ingested dose of pyridoxine is rapid, that significant elevations in blood levels can be achieved, that half or more of the dose is rapidly excreted by the kidneys, mostly as 4-pyridoxic acid, and that an appreciable fraction of the circulating amount penetrates into the central nervous compartment fairly rapidly. The results in Table VI suggest that an appreciable portion of the ingested pyridoxine is converted to circulating pyridoxal and pyridoxal phosphate, but not pyridoxamine. If the time courses of urinary excretion closely reflect changes in blood levels, multiple daily doses would be required to maintain elevated levels, particularly in intracranial fluids. More detailed information on these points would be desirable.

* We are indebted to Dr. George E. Boxer, of the Research Division, Merck and Co., Inc., Rahway, N. J., for these assays. Pyridoxal phosphate was determined by a modification of the method of Gunsalus *et al.*[63] Other assays were done by unpublished methods.

SUMMARY AND CONCLUSIONS

The evidence for a role of pyridoxine in neuronal metabolism and some of the problems encountered in attempting to elucidate the nature of this role have been reviewed. It is clear that pyridoxine in its coenzyme form is essential to the activity of a number of important enzyme systems in brain. It is also apparent that deprivation of pyridoxine results in epileptiform seizures in all species, including man. There is much evidence to suggest that deficiency of pyridoxine is associated with disturbances in the metabolism of neural lipids of myelin structures. The mechanisms responsible for these impairments of neuronal function remain obscure. Despite the incompleteness of knowledge in these regards, pyridoxine, as pyridoxal phosphate, must be accepted as essential to proper neuronal function and activity and as intimately concerned with the systems involved in the seizure process. Further investigation of these problems should not only provide data specifically related to pyridoxine deficiency, but also data of value to the general question of factors concerned with neuronal function and activity.

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The Neuropathology of Experimental Vitamin B_6 Deficiency in Monkeys

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THE GROUP of substances known as vitamin B_6 have been recognized as essential food factors since 1934;[1] and, since their isolation and synthesis, their particular importance in the nutrition of the nervous system has been established.[2] The first indications of their role in nervous metabolism were obtained entirely from animal experiments in which a deficiency of pyridoxine was found in rats,[3,4] chicks,[5] pigs,[6,7] dogs,[8] turkeys,[9] calves,[10] and ducks[11] to be regularly attended by convulsions; the pigs and dogs, in addition, suffered an ataxia of gait or weakness of the limbs. Later, a number of chance observations in the clinic gave support to the supposition that these vitamins were equally important in maintaining the integrity of the nervous system in man. This fact was first suggested in 1951 when a large number of infants receiving a commercial milk product which was proved to be deficient in pyridoxine developed convulsions.[15,16] The role of pyridoxine deficiency in producing convulsions in infancy was further demonstrated by Hunt *et al.*,[17] and by Snyderman and her associates[18] who actually produced convulsions in a feeble-minded infant by an experimental diet deficient in pyridoxine. Also, it was discovered that the neuropathy which occurred in the tuberculous patients receiving isonicotinic acid hydrazide (INH)[19–23] was due, not to an interference or metabolic antagonism of nicotinic acid,[19,22,24,25,26] but of pyridoxine.[23,27,28,34] The existence of a polyneuropathy due to pyridoxine deficiency was corroborated by the clinical investigations of Vilter and his associates,[29] who induced the symptoms and signs of peripheral nerve disease in humans by the administration of a pyridoxine antagonist, desoxypyridoxine.

The pathologic basis for these abnormalities in the central and peripheral nervous system has been the subject of very few studies. In dogs[8] and swine,[12,13] degenerative changes have been observed in the peripheral nerves, dorsal root ganglia, the posterior roots and the posterior columns of the spinal cord. These findings would explain the weakness and ataxia which have been seen in these animals. However, in the brains of these animals, as well as in those of rats,[14] no abnormalities were found. Hence, the cerebral process underlying the convulsive disorder remains unknown. It was this lack of precise data concerning structural alterations of the nervous system that encouraged us to reinvestigate the neuropathology of experimental vitamin B_6 deficiency. The purpose of this brief report is to summarize our preliminary observations on the first series of pyridoxine-deprived monkeys and dogs. All of the animal material was not completely studied in time for this symposium.

The observations herein recorded form part of a study of pyridoxine deficiency which was initiated at the Merck Institute of Therapeutic Research by Dr. Gladys Emerson and Dr. Charles W. Mushett. We are indebted to them for providing us with the nervous tissues from their animals for histologic examination. A more complete account of this experimental material, containing all the nutritional data as well as the general pathologic findings, will be published shortly by these authors. For the present it will suffice to mention only the

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general features pertinent to an understanding of the neuropathologic changes.

MATERIALS AND METHODS

Thirteen rhesus monkeys were used in this experiment. They were given a specially prepared diet and were under continuous observation for more than a year. In 12 of these monkeys the experimental period consisted of three parts. In the first period, lasting 86 days, the monkeys received a mixed natural ration, during which they grew well and thrived in general. In the second period, lasting 31 days, they received the vitamin B_6-deficient diet of Rinehart and Greenberg,[30] plus 3.5 mg of vitamin B_6 orally twice weekly; the monkeys continued to grow and remained healthy. In the third period, lasting 182–325 days, the pyridoxine was withdrawn from six of the monkeys. These latter monkeys soon began to lose weight and to appear sickly. Six other monkeys, serving as controls, were maintained on pyridoxine as before; and, with one exception to be mentioned later, they continued to thrive and grow at their previous rate. An additional monkey was rendered deficient by the administration of desoxypyridoxine for 47 days (100 mg/kg subcutaneously daily in two divided doses).

Five dogs were also used in the study (3 were "deficient" and 2 served as controls). The animals evidently could not survive on the pyridoxine-deficient diet, and when small amounts of pyridoxine were given symptoms of deficiency did not appear. Since no significant lesions of the nervous system were produced in the dogs, nothing more will be said about them.

Neither the deficient monkeys nor the deficient dogs showed convulsions or an ataxia of gait, although they were all weak, listless, and apathetic.

The entire brain and spinal cord of the animals were available for study, as well as cervical and lumbar ganglia and roots, and segments of the brachial and sciatic nerves. The material was fixed in 10 per cent formalin for at least two weeks, and suitable blocks were prepared for frozen, paraffin, and celloidon sections. Celloidon sections of the brain were stained by the Nissl technique for cell changes, and by the Loyez method for myelin. Similar sections of the cord were stained with Nissl and Luxol Blue. Paraffin sections of the spinal cord were stained with hematoxylin and eosin for cell changes, and by the method of Bodian for axis cylinder changes. Frozen sections of spinal cord, spinal ganglia, roots, and nerves were stained for fat and myelin with Oil-red-O-hematoxylin; paraffin sections of these tissues were stained for axis cylinders by the method of Bodian.

NEUROPATHOLOGIC CHANGES

The most prominent abnormality was seen in the nerve cells of the cerebral cortex. All of the deficient monkeys showed this change, although in varying degrees of severity, and it was also present in the monkey which had received desoxypyridoxine and in one monkey which, subsequent to a period of deficiency, had then received vitamin B_6 for over a year before being sacrificed.

The abnormality was most readily discerned in the large cells of the motor cortex—the cells of Betz—and to a lesser extent in the smaller pyramidal cells of the cortex. The affected cells appeared swollen and more roundish than usual, with eccentric nuclei, and varying degrees of loss of the Nissl particles. In addition, many of the large motor cells appeared dark, shrunken, and "spikey," and many of the smaller nerve cells in all layers of the cortex were unusually pale or showed a finely vacuolated cytoplasm (Fig. 1A). The nerve cells of the basal ganglia, subthalamus, and thalamus did not show swelling, chromatolysis, or eccentricity of nuclei.

In addition to changes in the Betz cells, in some of the deficient monkeys there was an increase in the number and size of the astrocytic nuclei (Fig. 1A). In one animal in particular there was a striking increase in the number and size of the nuclei of the protoplasmic astrocytes in all layers of the cortex and in the most superficial parts of the subcortical white matter beneath it. Several other deficient animals showed this change, but only to a slight degree. There appeared to be no parallelism between the Betz cell and astrocytic changes—in fact, in the animal with the most prominent

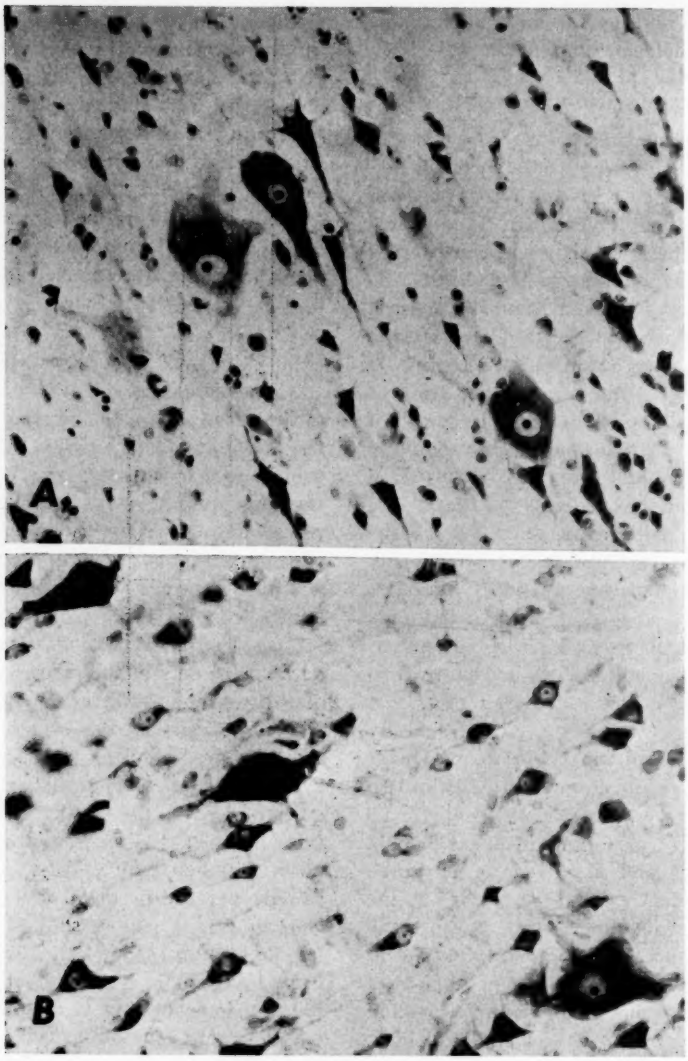

Fig. 1. A. The motor cortex of a pyridoxine-deficient monkey, showing swelling, chromatolysis, and eccentricity of the nucleus in the Betz cells, as well as the dark angular "spikey" cells. × 250.
B. The motor cortex of a nondeficient monkey, showing the normal appearance of the Betz cells. × 250.

disorder of motor cells, the astrocytes were regarded as normal.

In contrast to the cerebral cortex, the brain stem, cerebellum, spinal cord, dorsal root ganglia, anterior and posterior roots showed no consistent lesions (Figs. 2A and B). There was an isolated instance of pyramidal tract affection in one monkey. In the lower medulla, at the pyramidal decussation, and throughout the spinal cord in Oil-red-O-hematoxylin sections, there was seen a loss of medullated fibers and the presence of fatty macrophages in both pyramidal tracts.

The peripheral nerves were affected in all but one of the deficient animals. The unaffected monkey had been deprived of pyridoxine for 182 days—a shorter period than any of the others—which may account for the

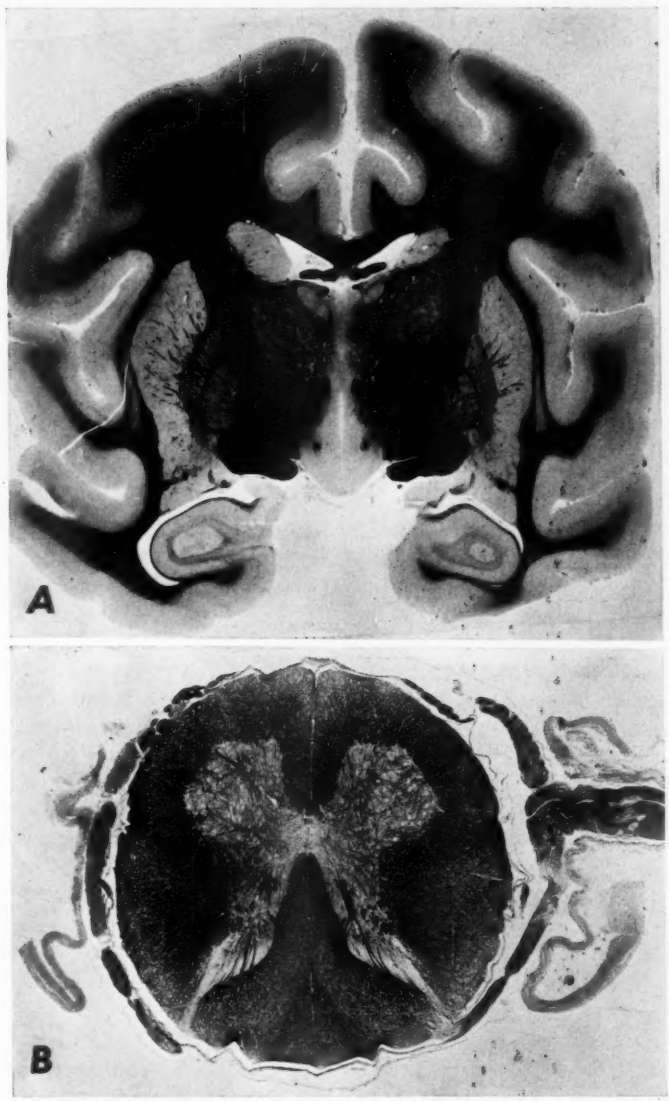

Fig. 2. A. A coronal section through the brain of a pyridoxine-deficient monkey, to show the intactness of the cortico-spinal tract projections. Myelin stain.
B. A transverse section of the thoracic portion of the spinal cord of a pyridoxine-deficient monkey, to show the intactness of the white matter. Myelin stain. × 10.

lack of nerve involvement. In general, the nerve lesions were slight and for the most part were limited to the myelin sheaths. In the involved nerves the myelin had degenerated in a patchy, segmental distribution. At times only part of a single internodal segment was involved and the myelin sheath was intact on each side of the lesion; in other nerves long stretches of myelin had disintegrated and were replaced by rows of fatty macrophages. It appeared that the myelin degeneration could begin in any part. Often the myelin degeneration was patchy; in one focus groups of adjacent fibers were affected (Fig. 3A). The

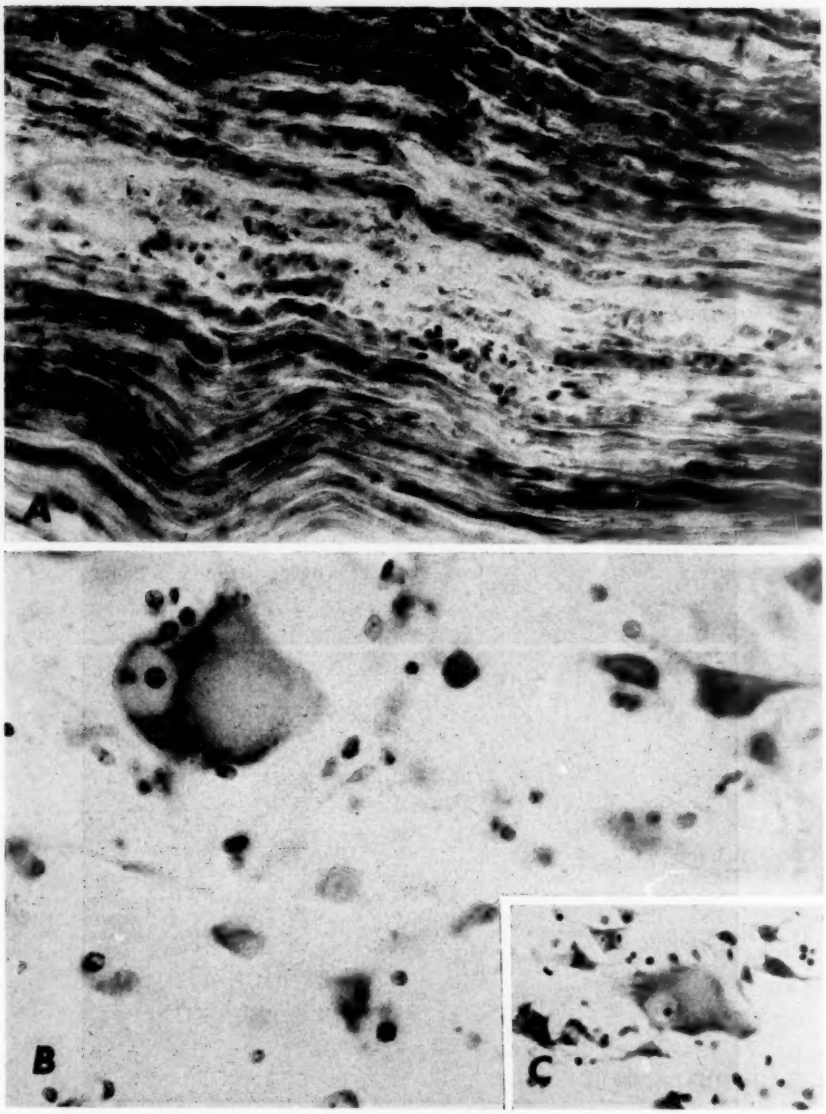

Fig. 3. A. A segment of the sciatic nerve of a pyridoxine-deficient monkey, showing an advanced degree of myelin disintegration and ingestion of myelin by fatty macrophages. Oil-red-O-hematoxylin. × 250.
B. Motor cortex from a patient with pellagra, showing the characteristic change in a Betz cell. × 450.
C. Inset. A Betz cell from the motor cortex of a pyridoxine-deficient monkey. × 250.

sciatic nerve was more affected than the brachial.

The axis cylinders were seen to be intact in most of the lesions. A striking exception occurred in the monkey which had received desoxypyridoxine; in this animal there was disintegration of many of the nerve fibers, both the myelin sheath and the axis cylinder being involved.

The anterior horn cells and dorsal root ganglion cells were unaltered. In none of the sections did the blood vessels appear abnormal.

Four of the control animals showed no significant neuropathologic changes. One control

animal, however, showed symmetrically placed destructive lesions of the brain stem, similar to those seen in human cases of Wernicke's disease. This animal was abnormal in many ways, for he failed to thrive and was subject to intercurrent infections. Since he was so different from the other monkeys, both controls and deficient, it was felt that he could not be suitably used for comparative purposes.

DISCUSSION

The interpretation of these pathologic observations depends to a large extent on the significance with which one regards the changes in the large motor cells of the cerebral cortex. These changes, comprising chromatolysis, swelling, and eccentricity of the nucleus, were so striking and departed so visibly from the normal that we are inclined to accept them as a significant pathologic change. In our own experience with experimental neuropathologic diseases such an appearance is unique. It was not encountered in the pyridoxine-deficient swine reported by Follis and Wintrobe,[12] or by Swank and Adams,[13] despite the presence in these animals of advanced peripheral nerve lesions. Zimmerman[31] states that this cell change has never been produced experimentally; nor are we aware of a description of this cell change in the literature on experimental pyridoxine deficiency or on the other B-vitamin deficiencies.

The question which has not yet been answered by our material is whether this swelling could represent a post-mortem artefact. All except one of the experimental animals died during the night and were not sacrificed, in contrast to the control group in which all but one of the animals were sacrificed. This may well be the explanation of the pyknotic, shrunken nerve cells which are known to increase in number and prominence as part of autolysis. However, we had not previously observed cell swelling under such circumstances. Aside from the problem of post-mortem artifact, the nature of our experimental material does not entirely exclude the possibility that inanition in general, rather than the specific lack of pyridoxine, is responsible for the cell change.

If, indeed, this nerve cell picture is a significant one, then the human counterpart which immediately suggests itself is the condition referred to as central neuritis. This cell change in the large motor cells of the cortex, comprising swelling, chromatolysis, and eccentricity of the nucleus, was first described in 1901 by Adolf Meyer;[32] since then it has been recognized as the distinctive cerebral lesion in pellagra (Fig. 3B). In rare instances this type of nerve cell alteration has been described in other states, such as chronic alcoholism, delirium tremens, Pick's disease, and chronic psychoses.[33] Despite its occurrence in isolated instances, this cell change is not specific for any of these states; it may reflect either a state of nutritional depletion which may occur in all of them, or be due to a lesion in a particular part of the neurone, i.e. the axis cylinder, and represent an "axonal reaction."

Although the cell changes in the deficient monkeys and in human pellagra are strikingly similar, they are nevertheless not absolutely the same (Figs. 3B and C). In pellagra the degree of eccentration of the nucleus may be greater, and the cytoplasm may appear lighter and less granular. The significance of such fine differences is difficult to judge.

The term "axonal reaction" is frequently used to designate a similar nerve cell change which occurs most frequently in the anterior horn cells when their axones are severed. This cell change probably is the consequence of a loss of nucleoproteins from the perikaryon through the damaged axone. It has never been decided whether or not the central neuritis of pellagra is the same as the axonal reaction and whether it is, in fact, dependent on injury to the axones of the Betz cells. One argument against this possibility is the frequency with which the motor tracts are interrupted in the brain and spinal cord without an axonal reaction appearing in their cells of origin. Moreover, in pellagra the cortical nerve cell changes have not always been associated with disease of the pyramidal tracts. Certainly the cortical cell change in the pyridoxine-deficient monkeys was independent of microscopically visible pyramidal tract injury —for in only one deficient monkey was there any evidence of a pyramidal tract lesion. It is

more likely that this represents a primary cytolytic degeneration of the whole motor cell, as suggested by Pearson;[33] or it may represent a cytoplasmic alteration to a purely biochemical abnormality in the axone which only reaches the stage of visible myelin degeneration in certain of the more acute or severe cases.

SUMMARY

The neuropathologic changes in pyridoxine-deprived monkeys are described. The most prominent abnormalities are seen in the large nerve cells of the cerebral cortex, which show swelling, eccentricity of nuclei, and loss of the Nissl particles. This cell change bears a strong resemblance to that seen in human pellagra.

The preliminary nature of these observations is stressed. The significance of the nerve cell change itself and the specificity of this change in pyridoxine deficiency are still in doubt; observations on considerably more experimental animals are required to settle these matters.

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Effects of Vitamin B_6 on the Central Nervous Activity in Childhood

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VITAMIN B_6 was first noted to be important to central nervous system metabolism in 1938 by Chick, who was doing deficiency studies in pigs. This finding has been repeatedly corroborated by later workers using rats,[2,3,7–9] dogs,[4] pigs,[5] and chicks.[6] Experiments have produced neuropathologic changes, abnormal behavior patterns, bizarre responses to stimuli, and convulsive seizures. Investigators have followed a number of procedures, including individual dietary deficiency, maternal dietary deficiency, antivitamin activity, and isoniazid toxicity. In almost all projects, the young of the species were shown to have the most labile central nervous system reactivity and the greatest susceptibility to vitamin deficiency and derangement.

VITAMIN B_6 STUDIES IN MAN

In 1950, Snyderman and co-workers demonstrated clinical evidence of nervous system effects in two children who had been fed a diet deficient in vitamin B_6.[10,11] Further support of these observations was gained during the 1951–53 incident of convulsive seizures appearing in infants who had been fed a commercial formula with a vitamin B_6 content of 60 μg/liter. Series of such patients were reported by Coursin,[12] Moloney and Parmelee,[13] Eliot,[14] May,[15] and Hansen and associates.[16] These cases definitely established a borderline need for vitamin B_6 intake which, if not satisfied, would produce clinical and electroencephalographic changes in certain infants.

In 1954 Hunt *et al.*[17] reported a well-documented case of pyridoxine dependency in a youngster who had recurrent convulsive seizures and progressive mental deterioration. Seizures were controlled by 2 mg of pyridoxine daily, although the mental retardation showed no improvement.

The recognition of demonstrable effects of vitamin B_6 on the human central nervous system has led to a review of previous studies and has initiated a number of new projects. Early workers had used pyridoxine with little success in a variety of conditions including paralysis agitans, Sydenham's chorea, amyotrophic lateral sclerosis, and the muscular dystrophies. One of the reports of the use of pyridoxine for nervous system disorders in children was published in 1944 by Stone,[18] who used it in doses of 30 to 50 mg intraspinally. His patients exhibited no adverse effects from the treatment, nor, on the other hand, did they seem markedly improved by it.

Another significant paper from 1944 is that of Kugelmass,[19] who reviewed the nutritional basis for nervous disorders in children. He specifically related vitamin B_6 deficiency to symptoms of weakness, irritability, nervousness, ataxia, and abdominal pain. Furthermore, he was impressed with the clinical response of these phenomena to treatment with pyridoxine.

Clinical testing for the possible value of pyridoxine in convulsive seizures was initially done by Fox and Tullidge.[20] They administered daily doses of pyridoxine ranging up to 100 mg to eight epileptic boys ranging in age from 14 to 15 years. These patients were inmates of an institution and were continued on their anticonvulsant medication along with the vitamin. This combined therapy was continued for four weeks without improvement. It was concluded that the vitamin was of no value in the treatment of epilepsy and it was abandoned.

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* Director of Research.

The next clinical report was by Ernsting and Ferwerda[21] in 1950. Pyridoxine in doses averaging 100 mg daily was given to 14 patients with petit mal seizures. Five of these patients were completely cured, while three others showed improvement. The remaining six exhibited no changes. The five with best results had no convulsions for from 10 to 20 months after treatment was started. Furthermore, it had been possible to discontinue pyridoxine after 8 to 12 months.

To date, there have been at least 25 additional articles in the literature suggesting that vitamin B_6 may be of value in abnormal nervous system states. Perhaps the most significant of these have been concerned with the use of antivitamins and with the effects of isoniazid toxicity. Vilter and his group[22] have published a number of papers on the results of desoxypyridoxine ingestion in man. Their findings with regard to the nervous system have been primarily confined to polyneuritis which improved on pyridoxine therapy.

Reilly and co-workers[23] have shown that convulsive seizures in animals and man resulting from the use of isonicotinic acid hydrazide respond well to pyridoxine therapy. The pyridoxine in intravenous doses of 200 to 400 mg is effective in preventing and controlling seizures due to hydrazides such as the semicarbazides. However, it is not certain whether this represents an actual poisoning of the nerve cell structure by the isoniazid or a secondary effect of rapid depletion of available pyridoxine through its chemical combination with the hydrazide to form a hydrazone that cannot be used and is excreted.[24]

The most recent publication in this field is that of Livingston, Hsu, and Petersen[25] who have just presented a series of 31 children with epilepsy of varied types who were given doses of 100 mg of pyridoxine daily. Treatment was continued for at least one month, with no evidence of improvement in any of the patients. Twenty-one of these patients subsequently responded to specific anticonvulsant therapy.

At the present time, there are two other projects of a similiar nature being carried out, by Dr. George Gammon[26] at the University of Pennsylvania, and by Dr. Curtis Marshal[27] at the Johns Hopkins Hospital. Their preliminary findings have not as yet provided any clear-cut conclusions. In addition to these studies, Dr. Ruth Baldwin[28] of the University of Maryland has had some success in the use of pyridoxine in several children with postencephalitic paralysis agitans.

RECENT OBSERVATIONS ON VITAMIN B_6 EFFECTS

The studies of pyridoxine effects on the central nervous system that have been carried out by the Research Institute of the St. Joseph's Hospital have been entirely on infants and children. This age group appears to provide the best material and has permitted a number of interesting observations.

Our initial studies from 1951 to 1953 revealed the syndrome of abnormal central nervous system activity that accompanies low vitamin B_6 intake during infancy. This includes increasing hyperirritability, gastrointestinal distress, increased startle responses, and convulsive seizures. There may be electroencephalographic changes present during actual periods of seizures, but there are no characteristic diagnostic patterns. Furthermore, during the interseizure tracings, no abnormalities are found. Both the clinical and electroencephalographic changes dramatically respond to pyridoxine therapy. The duration of the response may be conditioned by the individual need and the dosage and route of administration. Treatment with 5 to 10 mg orally will prevent symptoms for several days, but must be repeated to control the situation. On the other hand, in at least one case, the intramuscular injection of 100 mg appeared to correct the underlying pathophysiology for three months.

A follow-up study of the original 54 patients who had received the diet low in vitamin B_6 has been made.[29] Of the 28 subjects who had complete studies with electroencephalographic tracings, 20 were available for re-evaluation. None of these had experienced recurrence of seizures, nor had any shown evidence of mental deterioration. Their electroencephalograms were normal both on routine testing and in response to stimuli.

A survey of the more widely used sources of milk feeding for infants has been made with several important findings.[30] Values for vitamin B_6 content of milks were found to vary from 60 μg/liter to 610 μg/liter. The most important factor in this variation was the heat processing for sterilization. Excessive heating was noted to cause a definite decrease in actual vitamin B_6 content by microbiologic assay. Furthermore, biologic assay with rats brought out the point that even with feedings containing normal quantities of heat-treated vitamin B_6, normal growth was not obtained.[31] Apparently, the heat effect had in some fashion further reduced the ability of a given quantity of vitamin B_6 to be utilized, thus increasing the state of deficiency. The question has been raised as to whether further processing in formula preparation may be of some significance in contributing to subclinical or mild vitamin B_6 deficiency in infants.

With this thought in mind, an effort has been made to evaluate the use of pyridoxine therapy in cases of so-called infant colic or hyperirritability with gastrointestinal distress. To date, there is some evidence to suggest that such a relationship may exist. However, until better measurements of blood levels of the vitamin can be made, it is impossible to evaluate this study accurately.

Finally, for the past two years we have been administering test doses of 100 mg of pyridoxine intramuscularly to a wide variety of patients on whom routine electroencephalograms were being done. About 100 patients of all ages and with a wide variety of central nervous system problems, as well as normal subjects, were followed. A somewhat similar experiment was reported by Gozzano *et al.* in 1949.[32,33] They worked with adults and felt that vitamin B_6 produced an increase in amplitude with no change in rhythm in the waves within one minute after administration and persisting for one-half hour.

In our series, there were few instances in which there were any demonstrable changes, either clinically or by electroencephalogram, after the injection. However, in at least one specific case, there were a number of interesting changes that are worth presenting.

Case Report

M. S. was an apparently normal white girl who was first admitted at the age of two years with vague complaints of recurring fevers. Routine studies were negative except for a positive 1:320 titer for typhoid. She was given a course of chloramphenicol and remained afebrile. Several weeks after discharge, she began to walk peculiarly, with some evidences of spasticity. She was followed by the orthopedists with little improvement. When seen again at two and a half years, her walking had become much less co-ordinated; she showed evident mental retardation with poor speech; and she appeared to have had some increase in the size of her skull.

Pneumoencephalography revealed evidence of moderate enlargement of both lateral and the third ventricles with very little cerebral cortex visualized on x-ray. Spinal fluid elements were normal. The electroencephalographic tracing was normal for her age except for some asymmetry with occasional high slow waves which appeared at times arising from either right or left hemisphere.

Craniotomy was performed, exposing a severe non-communicating hydrocephalus with block of the aqueduct of Sylvius. This was made up of fibrous tissue that probably represented a postencephalitic process. It was technically impossible to open the communications of the fourth ventricle, so a tube was used to bypass the fluid from the left lateral ventricle into the basal cistern. Poor drainage from this area necessitated a second operation in order to shunt spinal fluid from the subarachnoid space at the lumbar level into the peritoneum. The postoperative course was difficult and there was a slow return to consciousness and full activity over a period of 30 days. However, when improvement did begin, progress was rapid. During the subsequent 15 weeks, the child definitely was more alert and her gait showed better co-ordination and balance. Her mental ability greatly increased, with accompanying improvement in speech. The whole picture was one of continued change toward the expected capacities for her age.

Suddenly, 19 weeks after her operation, she became comatose. Physical examination

showed loss of reflexes and complete flaccidity of musculature. There was no response to any stimuli. Her temperature was normal and there was no evidence of infection anywhere in the body. Spinal tap showed 33 leukocytes/cu mm, 14 mg of protein, and 88 mg of sugar per 100 ml. The bypass tube was found to be patent, and there was no increased intracranial pressure. Shortly after admission, she developed generalized convulsive seizures that were accompanied by cyanosis, apnea, and rapid pulse.

Electroencephalographic tracings during this time revealed gross abnormalities by both monopolar and bipolar techniques. Fundamental frequencies varied from 1 to 3 per second to 5 to 7 per second, with amplitudes of 100–200 μv. The picture was one of generalized dysrhythmia with no specific definable characteristics. Moments of seizures produced tremendous deviation of pens, obliterating any measurable patterns. The patient did not respond to pain, light or sound stimuli.

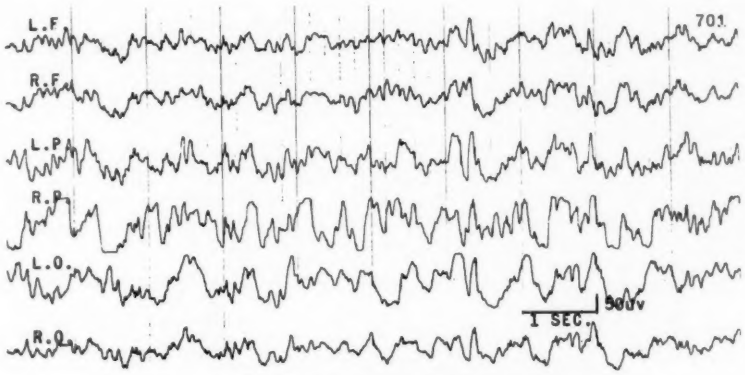

Fig. 1. Abnormal tracing recorded during coma.

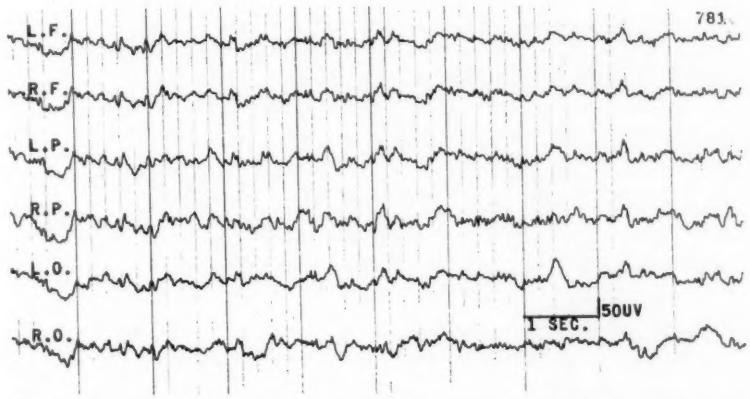

Fig. 2. Response within 1 minute after first intramuscular injection of 100 mg of pyridoxine.

Recording was carried out for 15 minutes and showed continuous patterns such as are seen in Figure 1. A syringe injection was made to obviate any false reaction to this stimulus. One hundred mg of pyridoxine was then injected intramuscularly with continuous recording. Within 60 seconds, there was a remarkable alteration in the tracing to an almost normal pattern for this age group (Fig. 2). The patient became wakeful and moved about with some response to light and pain but none

to sound. Convulsive episodes completely disappeared. Three minutes later she lapsed into sleep with comparable patterns in her tracing (Fig. 3). This condition persisted for two hours and fifty-two minutes. During this time, the patient could be aroused by stimuli, and move about, in contrast to her earlier comatose state. Gradually, the tracing showed reversion to the original pattern with reappearance of the previous abnormalities (Fig. 4). Clinically, she again became stuporous and did not react to stimuli. This regression took about three minutes once it began. The pattern of coma was followed for ten minutes and then a second intramuscular injection of 100 mg of pyridoxine was given. Within one minute, this entire procedure was repeated (Fig. 5).

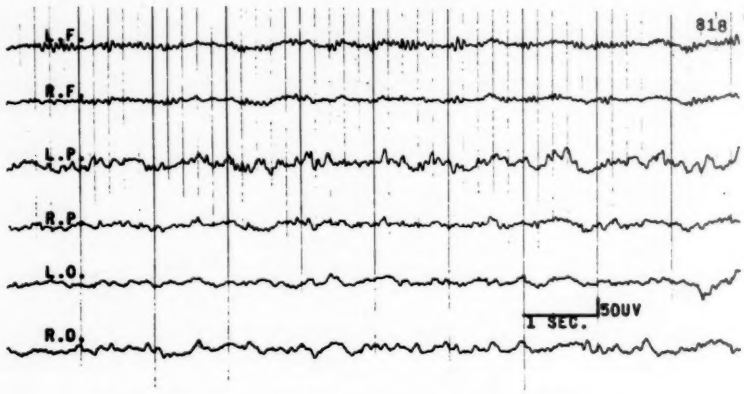

Fig. 3. Response within 3 minutes after injection of pyridoxine.

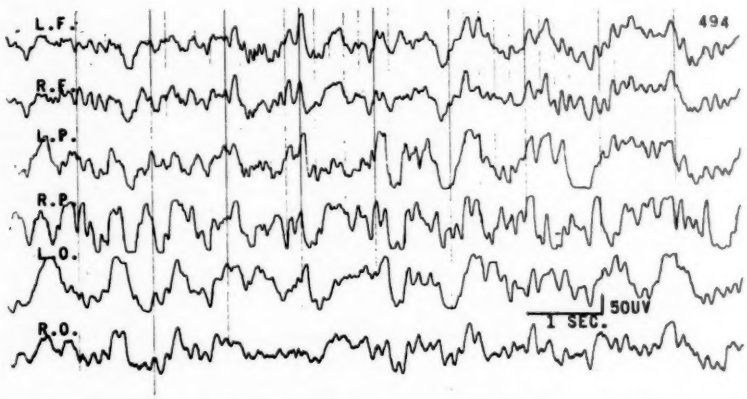

Fig. 4. Regression of tracing to original abnormality after 2 hours and 52 minutes.

Following this tracing, the patient was returned to Children's Ward where she was maintained on tube feeding of an adequate diet and supplemental vitamins. She was given 100 mg of pyridoxine intramuscularly four times a day for five days. During this time, there were no convulsive seizures. The clinical course continued to be one of occasional periods of wakefulness with response to stimuli interspersed with bouts of complete coma. On the sixth day, it was not possible to arouse her and the pyridoxine therapy was discontinued.

A second electroencephalographic study was made one week after the original one. Again the grossly abnormal patterns were evident, with predominant slow waves of 1 to 3 per second and amplitudes exceeding 200 μv

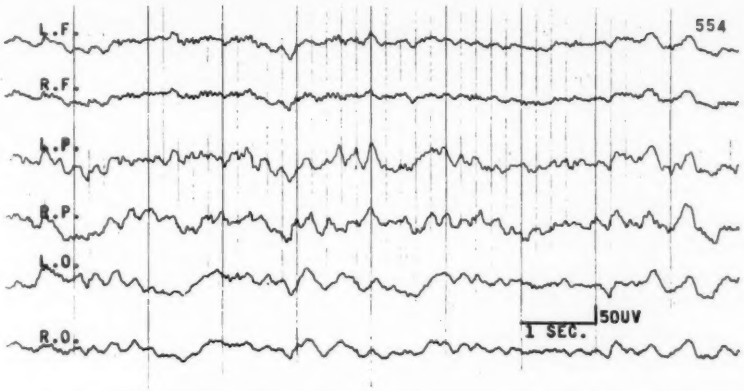

Fig. 5. Response within 1 minute after second intrasmuscular injection of 100 mg of pyridoxine.

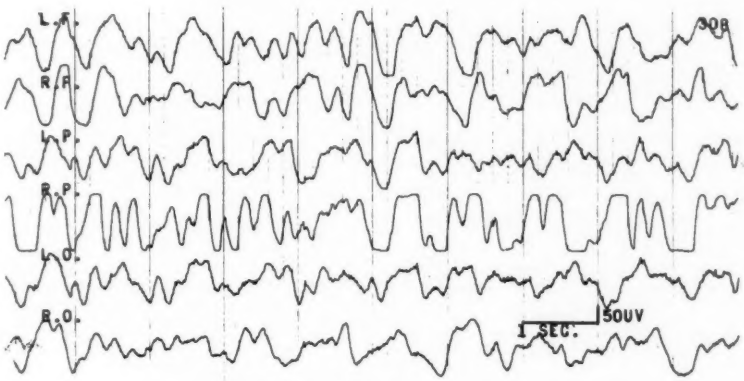

Fig. 6. Second abnormal tracing recorded one week after first tracing during coma. Monopolar technique.

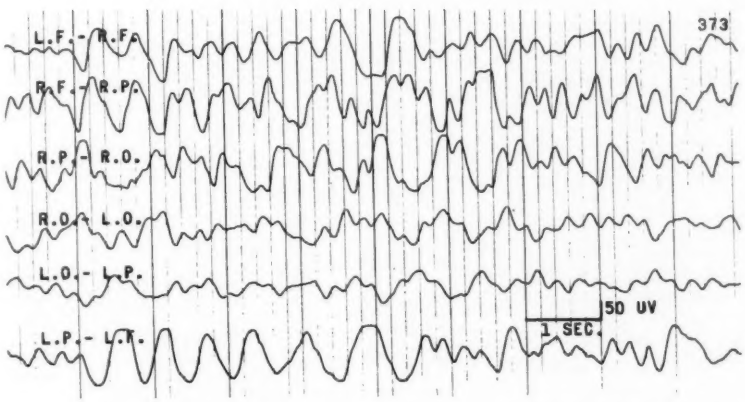

Fig. 7. Second abnormal tracing recorded one week after first tracing during coma. Bipolar technique.

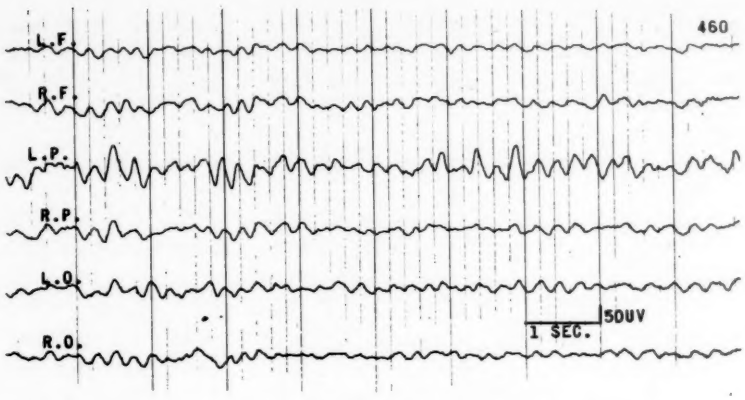

Fig. 8. Slow response to intramuscular injection of 100 mg of pyridoxine 5 minutes after injection.

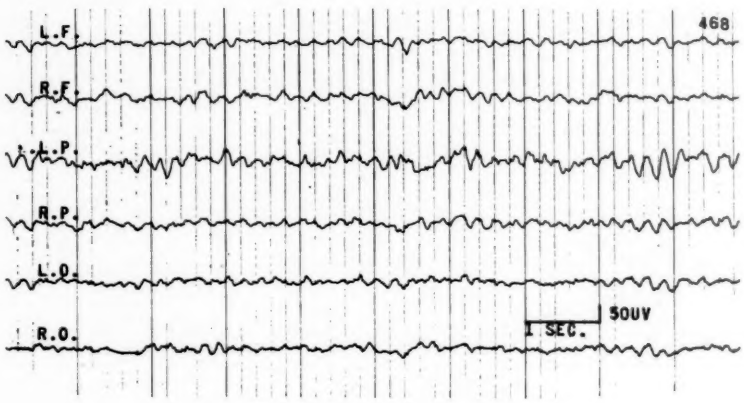

Fig. 9. Completed response to pyridoxine 6 minutes after injection.

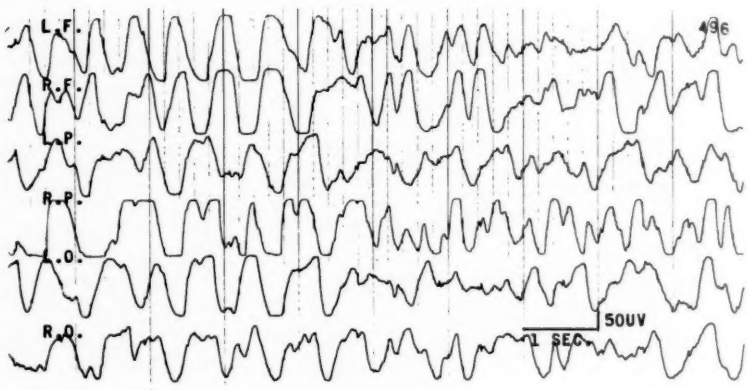

Fig. 10. Reversion to abnormal coma tracing after 6 minutes of stable pattern seen in Figure 9.

(Figs. 6, 7). There was no response to stimuli or to a placebo. One hundred mg of pyridoxine was given intramuscularly with no detectable response for five minutes. The tracing then began to change, with a marked decrease in amplitude and increase in frequency to 6 to 8 per second (Fig. 8). Within two more minutes, the change to one of significant improvement was complete (Fig. 9). The time interval for this new pattern was short-lived, being of only six minutes' duration, and there was a gradual reversion to the original grossly abnormal pattern (Fig. 10).

Since that time, the patient has shown progressive deterioration, and has become a severe cerebral palsy problem. She has had no convulsive seizures, but has remained comatose with no response whatsoever. Her flaccidity has given way to a rigid spasticity with markedly hyperactive reflexes, constant Babinski signs, opisthotonus, and fixation of the eyes. Her life has been sustained entirely by tube feeding and expert nursing care. Her spinal fluid, blood count, urinalysis, and blood chemical studies are normal. Electroencephalographic tracings show constant abnormalities of from 1 to 3 per second waves of 200 μv amplitude. There is now no response to pyridoxine in doses up to 250 mg given intramuscularly.

Throughout this entire period of hospitalization, and particularly during the electroencephalographic test periods, blood samples were drawn, separated, and quick-frozen for future study. Microbiochemical determination for pH, carbon dioxide, oxygen, chloride, calcium, phosphorus, sodium, magnesium, blood sugar, and protein fractionations did not show any change that could be interpreted as being contributory to this phenomenon. Tryptophan loading, mineral retention, and pyridoxic acid tests were not done. As a matter of fact, it is doubtful, until there are better means for directly determining vitamin B_6 in the blood, that one will be able to assess these problems correctly.

SUMMARY AND CONCLUSIONS

Considering the information at hand, it would appear that vitamin B_6 plays a definite part in central nervous system metabolism. Under gross conditions of deficiency, deprivation, antivitamin activity, and isoniazid toxicity, clinical and electroencephalographic changes are to be expected and are readily correctible. However, the more challenging problems lie in the coenzymatic and synergistic roles of vitamin B_6 in cellular metabolism.

At the present time, there is much speculation as to how the phenomenon just discussed transpires. The significant features of our observations have centered about the rapidity of response and the dramatic correction of grossly abnormal electrical discharges from the brain with simultaneous clinical improvement. One may deduce that these alterations have taken place under the influence of relatively small amounts of pyridoxine, since it is doubtful that time would have permitted the absorption of much of the routine 100 mg injected intramuscularly. The quantity of the vitamin picked up by the body apparently contributes a necessary key to biochemical reactions within the brain cell, permitting either a release mechanism or an inhibitory one to function. It is conceivable that this may involve all of the effects of vitamin B_6 on fat, carbohydrate, and amino acids. However, it is more likely that the tremendous energy transfers involved arise primarily from the control of phosphate bonds and the utilization of adenosine triphosphate in the correlated cycles of glucose and amino acid metabolism. It is hoped that with improved techniques for measuring the constituents of these reactions, as well as blood levels of vitamin B_6, it will be possible to understand better the intracellular relationships involved, and perhaps reveal the true origin of many nervous system disturbances.

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For discussion see page 363.

DISCUSSION

DR. CHARLES MAY (Iowa City, Iowa): Insofar as historical aspects are concerned, once again we have seen how important basic observations are to the development of new chapters in science. Once these initial observations are made, it is rather easier for many of us to follow through with appropriate techniques and erection of new hypotheses. For some of the acceleration of interest in vitamin B_6 we are indebted to ordinary general practitioners, who, in the course of their practice, noted that an infant food was singularly involved in the occurrence of convulsions. It was their observations that needed explanation, and the explanation was only gradually forthcoming. I mention this because those of us who are teaching clinical medicine might still encourage the physicians in practice to appreciate their important position, their unique position, for making observations which may actually usher in what sometimes seem to be more sophisticated chapters in the development of a subject.

Other items which have interested me are: the difference between species in the appearance of manifestations of pyridoxine deficiency; the progressive sequence of events which occurs in a given species; the partial adequacy of antagonists to simulate deficiency of vitamin B_6; and the implications of various portions of the molecule for the development of particular manifestations of deficiency or of the antagonism. Furthermore, I should have liked to have heard from someone as to how we might more effectively attack the perplexing problem of determining requirements of vitamins in infants and adults, when faced with some of the difficulties of assay and of the role of intestinal flora in this matter. I think we are all accustomed to the paradoxical lack of correlation at time between morphological change and functional change. I believe that this is not in any way a discredit to the morphologist, for very frequently it is our inability to observe the corresponding functional change which makes these things difficult to correlate.

DR. E. E. SNELL (University of Texas, Austin, Texas): I do think there has been too much attention paid in the past to enzymes that do not reflect sensitivity to vitamin B_6 deficiency in intact animals. The first enzymes to be shown to require vitamin B_6 phosphate were certain decarboxylases and some of the transaminases. Many of these are relatively insensitive to restriction in the dietary intake of vitamin B_6. As Dr. Tower has pointed out earlier this morning, some of the enzymes more sensitive to vitamin B_6 deficiency include those concerned in the metabolism of cysteine and perhaps other sulfur amino acids. It should be useful to examine such enzymes, e.g. in serum, for sensitive methods of detecting vitamin B_6 deficiency, rather than restricting such investigation to the transaminases. One question occurred to me at the close of the last presentation, and this is whether pyridoxal or pyridoxamine had been tried in the individual who failed to respond to 400 mg of pyridoxine administered every three hours? When one administers pyridoxine it must be recognized that the availability of this substance may be limited in some individuals by their ability to transform it into an enzymatically active form, and in such cases it should be only caution at least to try directly the more immediate precursors of pyridoxal phosphate, or even the coenzyme itself. Even when pyridoxal is administered, its activity may be limited by the rate at which it is converted to pyridoxal phosphate. On the other hand, the activity of pyridoxal phosphate might be limited if transfer of this phosphorylated compound through mammalian cell membranes was limited.

Some Metabolic Effects of Vitamin B_6 in Vivo

By E. W. McHenry, PH.D., F.R.S.(C.)*

THIS discussion of biochemical changes in the vitamin B_6-deprived rat commences with reference to a simple set of observations with which we are all familiar. Within a few days after the start of the deprivation the rat shows a decreased appetite, consequent to which there is a progressively more evident failure to increase body weight. A control, receiving the vitamin but pair-fed with the deprived rat, exhibits no loss of appetite and gains weight slowly, so that after several weeks there is a significant difference in weight between the deprived and the control rats. Carcass analyses have shown that the difference in weight is accounted for entirely by difference in content of fat and water; the protein contents are nearly equal.[1]

This set of observations is not unique for vitamin B_6 deprivation but is shown in nearly all nutrient deficiencies. In the case of vitamin B_6, we have no explanation for the decrease in appetite. For some years the anorexia in thiamine-deprived rats has been explained by the use of early observations of gastric hypotonus, observations which could not be duplicated in some subsequent studies.

An elevation in fasting blood sugar or a prolonged rise after food ingestion could explain the decrease in appetite. Neither of these changes has been observed so far. In fact, a decreased fasting blood sugar has been reported from our laboratory.[2]

Although the vitamin B_6-deprived rat has less body weight and less fat than the pair-fed control, the oxygen consumption per unit of body weight is equal.[3] The consumption per animal is greater for the control than for the deprived rat because of the difference in body weight. The data for oxygen consumption have failed to explain the difference in body weight. Studies on food absorption in several laboratories[4,5] have been similarly fruitless in explaining the body weight difference, since no failure in absorption was evident in the deprived animal.

PROTEIN AND FAT METABOLISM

We have not seen any failure in protein maintenance or synthesis in vitamin B_6-deprived rats. The blood proteins are not decreased;[5] the total protein content in the carcass is not lessened, although the deprived animal is in somewhat less positive nitrogen balance.[5] A negative nitrogen balance has not been observed even in severe deficiency. Neither does the deprived rat show any lessening in the ability to regenerate liver protein after partial hepatectomy.[6] We have concluded that vitamin B_6 deficiency in the rat does not impair protein maintenance or protein synthesis. It should be noted that the less positive nitrogen balance is due to an increased excretion of urea and that liver slices show an increased rate of urea formation.[7]

Reference has been made to the difference in fat content between the deprived rat and the pair-fed control. No real difference in the spectrum of fatty acids has been seen other than some increase in the proportion of highly unsaturated fatty acids in the deficient animal.[8] The proportion of phospholipids is also increased. Studies with C^{14}-labeled glucose suggest that the deprived rat's ability to synthesize fat from carbohydrate is not impaired.

CARBOHYDRATE METABOLISM

Dr. J. R. Beaton has recently reported his observations on the effects of vitamin B_6 depri-

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vation on carbohydrate metabolism. These observations are summarized briefly. As early as five days after omitting the vitamin from the diet, rats show a reduction in fasting blood sugar, and the discrepancy in blood sugar between deprived and control rats is accentuated as the deficiency progresses.[2] The fasting blood levels of pyruvic and lactic acids are also decreased. The amount of hepatic glycogen is diminished, as is the lactic acid dehydrogenase activity of liver homogenates. Beaton also found significant elevations in inorganic phosphorus and glutathione in blood and livers from deprived rats.[9] No significant difference was found in glucose utilization by muscle, nor in liver cytochrome oxidase activity. Liver aldolase activity, however, was definitely decreased. The plasma alkaline phosphatase activity was markedly increased in deprived rats.[10] All of these findings led to the conclusion that vitamin B_6-deprived rats exhibit alterations in carbohydrate metabolism.

ENZYMATIC ACTIVITY

It is widely accepted that vitamin B_6 acts as a coenzyme for one or more transaminases, for amino acid decarboxylase, and presumably in the dehydration of hydroxyamino acids and in the desulfhydration of sulfur amino acids. A recent review[11] contains the statement that "Pyridoxal-5-phosphoric acid can be considered as almost generally required for enzymatic reactions involving the nonoxidative degradation and interconversion of free amino acids." This explanation of the function of vitamin B_6 is generally accepted but, unfortunately, there are still questions regarding biochemical changes in the intact animal. Does this function explain the various biochemical alterations which have been observed in the intact animal? Does the accepted function explain the dermatitis found in the deprived rat? Does this explain the production of atherosclerosis in the deprived monkey?

From these questions stem a secondary set of problems: Is the evidence regarding the relation of vitamin B_6 to transamination so clear-cut that it must be accepted? What purposes are served by transamination in the metabolic processes of the intact animal? Furthermore, how many of the reported biochemical abnormalities are specific for vitamin B_6 deficiency?

In a study[1] of the chronological development of biochemical changes in the vitamin B_6-deprived rat, we found that transaminase activity in the liver did not decrease as the deficiency developed but remained fairly constant. The transaminase activity in the livers of control rats increased and seemed to keep pace with body weight. If we had had no transaminase estimations available except those at the end of the experiment, we would have concluded, probably, that transaminase activity had decreased in the deficient rat. Several other discordant notes may be added. The total vitamin B_6 content of the liver attains a minimal level comparatively early in deprivation. Administration of deoxypyridoxine does not lessen transaminase activity in the liver but actually causes an increase.[12]

TRANSAMINASE

We have recently extended observations on the alanine-glutamic and aspartic-glutamic transaminase activities in rat liver, and in parallel experiments have compared the effects of several B-vitamin deficiencies. Separate observations were made on male and female rats. In each deficiency the selected time of measurement was when the deprived animals had reached a plateau in body weight. Starting with young rats, there was an initial period of weight increase. Following an impairment of appetite, body weight was constant for several days. Deficient animals and corresponding pair-fed controls were killed for enzyme and other studies. This arrangement was followed in the hope that the various deficiencies would be comparable in degree at that period. It should be noted that rats deprived of various vitamins reached the chosen stage at different intervals.

In the case of alanine-glutamic transaminase, deficient male rats showed the failure to increase which had been noted previously, but the deficient females had a real decrease. The data for this enzyme are given in Table I. Rats deprived of any one of four other B vitamins (thiamine, riboflavin, pantothenic acid,

TABLE I

Hepatic Alanine-Glutamic Transaminase in Deficiencies of Several B Vitamins

Status	Pyruvate (μl/g/hr)		
	Males	Females	Mean
Initial	40	48	44
Thiamine-deficient	58	63	60
Riboflavin-deficient	88	56	72
Vitamin B_6-deficient	38	34	36
Pantothenate-deficient	60	64	62
Biotin-deficient	65	63	64
Average paired controls	52	51	52
Ad lib controls	52	65	58

or biotin) showed increases in alanine-glutamic transaminase, the most marked increase being evident in riboflavin deprivation.

Values for aspartic-glutamic transaminase showed a lesser change (Table II). Vitamin

TABLE II

Hepatic Aspartic-Glutamic Transaminase in Deficiencies of Several B Vitamins

Status	Pyruvate (μl/g/hr)		
	Males	Females	Mean
Initial	124	94	109
Thiamine-deficient	126	121	123
Riboflavin-deficient	139	130	135
Vitamin B_6-deficient	101	99	100
Pantothenate-deficient	117	104	111
Biotin-deficient	135	129	132
Averages of paired controls	123	112	118
Ad lib controls	92	125	109

B_6-deficient male rats had a slight decrease, while rats deprived of either riboflavin or biotin exhibited increases in this enzyme. It had been reported previously[13] that aspartic-glutamic transaminase was insensitive to changes in vitamin B_6 nutriture.

Contrasting the deficiencies of five separate B vitamins, it is evident that alanine-glutamic transaminase either decreases or fails to increase in vitamin B_6 lack. In four other deprivations the hepatic activity of this enzyme is increased. This difference provides support for the conclusion that vitamin B_6 deficiency has an opposite effect on alanine-glutamic transaminase from that found in four other deficiencies (thiamine, riboflavin, pantothenic acid, and biotin). Observations on aspartic-glutamic transaminase show the two opposite trends, but not so definitely.

TABLE III

Ratios of Alanine-Glutamic Transaminase to Body Weight in Several B-Vitamin Deficiencies

Vitamin		Deficient	Pair-fed control
Vitamin B_6	Males	0.19	0.21
	Females	0.21	0.36
Thiamine	Males	0.31	0.21
	Females	0.45	0.30
Riboflavin	Males	0.49	0.28
	Females	0.36	0.31
Pantothenic acid	Males	0.27	0.18
	Females	0.37	0.26
Biotin	Males	0.26	0.22
	Females	0.33	0.23

In a previous report[13] from our laboratory a sex difference in transaminase activities was noted. Some differences in the sexes were also found in the more recent observations. From work on hamsters, Shwartzman and Hift[14] concluded that hepatic transaminase activity increases with age. In one experiment we noted a confirming observation. However, we have had the impression that there is a relation between the amount of hepatic transaminase activity and body weight, at least in normal animals. If such were the case, the ratio of transaminase activity to body weight should be fairly uniform. A deviation from such a normal ratio could be a better indication of a change in transaminase activity than the absolute activity. At least, this ratio would allow for a possible correlation of transaminase with body weight. The ratios for group averages in the recent observations are shown in Table III. Two conclusions may be drawn from these ratios: (1) some sex difference is evident; (2) transaminase alterations in vitamin B_6-deficient rats are different from alterations in individual deficiencies of four other B vitamins. The observations, then, are in accord with the generally accepted view that vitamin B_6 is related to transamination.

SPECIFICITY

Before we can consider how the involvement in transamination could explain any of the various biochemical alterations seen in vitamin B_6-deprived rats, it is necessary to decide whether various biochemical changes

are specific for this deficiency. There is no point in attempting to correlate a function with observed alterations unless the function and the alterations are specifically caused by the nutritional deficiency.

It has been noted that the rat deprived of vitamin B_6 shows a lessening in appetite, a failure to gain weight, and a decrease in fat storage (or at least less fat storage than a pair-fed control). None of these effects of the deprivation is specific for a lack of vitamin B_6. All of them have been observed in rats deprived of almost any nutrient. Recently we have made observations on several biochemical aspects of five B-vitamin deficiencies. At the particular stage of insufficiency used we could find no evidence that changes in fasting blood sugar, in fasting blood urea, or in liver glycogen were characteristic for any one of the deficiencies. While many other aspects need to be studied, it should be noted: (1) that B-vitamin and possibly other nutrient deficiencies present a common picture with regard to some biochemical alterations; (2) that the only alteration observed by us so far to be different in vitamin B_6 deficiency from other B-vitamin deprivations concerns alanine-glutamic transaminase.

DISCUSSION

There are, however, discordant aspects with regard to the relation of vitamin B_6 to transamination. Hepatic transaminase activity does not keep pace with the total vitamin B_6 content of the liver and tissues. This could be explained by conjecturing that the enzyme activity keeps pace with the amount of pyridoxal, but not with the quantities of other vitamin B_6 compounds. Such a conjecture would imply a lack of convertibility even in a stage of deprivation. Rats given deoxypyridoxine and deprived of the vitamin develop dermatitis rapidly but show an increased transaminase activity in liver homogenates. Either an impairment in transamination has no relation to the dermatitis, or else transamination is not the principal role of the vitamin. Changes in appetite, in carbohydrate metabolites, and in body composition may not be specific for vitamin B_6 deficiency, but they are clearly evident before a decrease in transaminase can be detected.

If we assume, as is commonly done, that the role of pyridoxal as the coenzyme for transamination is the main function of the vitamin, it is useful at the present stage of our knowledge to consider the purposes which may be served by the vitamin acting in transamination in the intact animal. It may be that transamination is a pathway in amino acid conversion to carbohydrate and, through carbohydrate, to fat. A decrease in transaminase activity would lessen this type of amino acid degradation and force greater amounts of amino acids to be catabolized in other ways, for example, by deamination. In this way we could explain the increased production of urea, and, possibly, an unusual pathway for the degradation of tryptophan. If transamination is one of several customary methods for handling some carbohydrate metabolites, we could partially explain the presence of an increased amount of pyruvic acid and perhaps of lactic acid, both of which have been found in augmented amounts in the blood of fasting, deprived rats.

In a discussion of biochemical changes in the intact animal two admissions are necessary at present. I have presented a picture showing a lack of essential information and of confusion. A partial account of biochemical lesions is available, but the number of these alterations which are really specific for vitamin B_6 deficiency is not known. The lessons to be drawn are obvious.

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Evidence for Abnormal Vitamin B_6 Metabolism in Pregnancy and Various Disease States

By M. WACHSTEIN, M.D.*

VITAMIN B_6 was recommended for the treatment of nausea and vomiting of pregnancy as far back as 1942 on a purely empirical basis.[1] While some investigators[2,3] reported excellent therapeutic results, others were more skeptical.[4] Evidence for a relative pyridoxine insufficiency in this condition was presented in 1949 by McGanity and associates.[5] These workers showed that subjects with hyperemesis gravidarum exhibit an abnormal response of blood urea levels following the administration of alanine, which could be corrected by pyridoxine.

Since the original discovery of vitamin B_6, many data concerning the fundamental importance of this vitamin for various metabolic processes have been accumulated.[6] Some of this knowledge has become useful in clinical investigation. Thus it has been possible to examine the question of abnormal vitamin B_6 metabolism in pregnancy, as well as in other conditions, in a more objective manner.

INTERMEDIARY METABOLISM

Vitamin B_6 is involved in the intermediary metabolism of tryptophan, since the breakdown of kynurenine and 3-hydroxy-kynurenine, both intermediary tryptophan metabolites, is dependent on the enzyme kynureninase. This enzyme needs vitamin B_6 for its proper function. In its absence, xanthurenic acid is formed in increased amounts and excreted in the urine.[7] This substance can be determined quantitatively by a simple colorimetric procedure. Following the ingestion of 10 grams of DL-tryptophan[8] (or 5 grams of L-tryptophan[9]) normal individuals excrete only small amounts of xanthurenic acid in the urine. In contrast, in vitamin B_6 deficiency the excretion of this substance is markedly increased, although xanthurenic acid does not appear in the urine of human beings in measurable amounts unless tryptophan is given in excessive amounts.

Sprince and his co-workers[10] applied the tryptophan load test to normal women and to women in the last trimester of pregnancy. Total xanthurenic acid excretion was not measured quantitatively, since only its concentration in the morning specimen before and following the test was determined. Nevertheless, a higher concentration of xanthurenic acid was found in the urine of women in whom the pregnancy was complicated by toxemia or other diseases, while in uncomplicated pregnancies the concentration was not different from that of normal controls. Independently, Vandelli in Italy also described a disturbance of tryptophan metabolism in pregnancy.[11]

METHODS AND MATERIAL

In our experiments, xanthurenic acid excretion was determined quantitatively using the method of Rosen, Lowy, and Sprince,[12] with some modifications, in a 24-hour specimen following the ingestion of 10 grams of DL-tryptophan.[13] Twenty-four normal controls excreted between 0 and 38 mg (average: 12.5 mg). Twenty-five patients with various diseases showed excretion values between 0 and 74 mg (average: 21 mg). The urine of 100 women at term contained between 0 and 813 mg (average: 195 mg). However, only 5 of these 100 women excreted amounts in the range found in normal controls (0–32 mg). A

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significant increase in xanthurenic acid excretion was first detected in the 12th to 14th week of pregnancy.

Twenty-one patients, with typical symptoms of toxemia of pregnancy, were given the tryptophan load test. They excreted between 94 and 666 mg of xanthurenic acid (average: 300 mg). This is a significantly larger amount than occurs in women with uncomplicated pregnancies.[14]

The influence of vitamin B_6 administration on the abnormal tryptophan load test was then determined in several experiments: (1) Several women in various stages of pregnancy were first tested for xanthurenic acid excretion and then treated with pyridoxine hydrochloride. It was found that following the ingestion of 25 mg of pyridoxine for 3 to 5 days the excretion of xanthurenic acid disappeared. When the test was repeated 10 to 15 days later, the abnormal amounts of xanthurenic acid reappeared in the urine.

(2) Women were tested one day after delivery and the test was repeated within 48 hours. When the tests were repeated without any vitamin B_6 supplementation or following the administration of a mixture of 5 mg of thiamine hydrochloride, 7 mg of riboflavin, and 50 mg of nicotinic acid, no significant differences were noted. These vitamins were chosen since an increased xanthurenic acid excretion has been reported not only in vitamin B_6-deficient rats, but also in those with induced thiamine and riboflavin deficiency.[15] In pyridoxine-deficient rats, on the other hand, xanthurenic acid excretion can be depressed by the administration of nicotinic acid.[16] When pyridoxine hydrochloride was given 30 minutes before the second load test to these experimental subjects in the amounts of 2.5, 5, 10, and 15 mg, respectively, even the smallest dose led to a highly significant depression of xanthurenic acid excretion.

(3) Following the administration of 25 mg of pyridoxine hydrochloride given at different time intervals either before or after the second tryptophan load test, from 24 hours preceding to 1 hour following the second test dose, a significant decrease of xanthurenic acid excretion was observed.

(4) In this series the influence of long-term vitamin B_6 administration was investigated. Xanthurenic acid excretion following the administration of 10 grams of DL-tryptophan was measured as early as possible in pregnancy, and the women were instructed to take various daily doses of pyridoxine. During treatment, load tests were repeated whenever possible. While daily oral supplementation of their usual food with 2.5 mg of pyridoxine had no significant influence, the effect of 5 mg was quite distinct. Complete suppression of the abnormal xanthurenic acid excretion was noticed in the group of pregnant women who were given 10 mg daily. Similar findings have been recently reported by Zartman and associates.[18]

OTHER STUDIES

The amount of 4-pyridoxal acid, the main metabolic end-product of vitamin B_6, was determined fluorometrically in the urines of pregnant women. No difference was found between pregnant women and non-pregnant control subjects.[19] Others, however, have found lower values in pregnant women.[20] For further evaluation, the vitamin B_6 load test of Sarett[21] was therefore applied with some modification. Following the ingestion of 25 mg of pyridoxal hydrochloride, urine was collected for an eight-hour period and 4-pyridoxic acid was quantitatively determined. The urines of 20 normal control subjects contained an average of 11.55 mg of 4-pyridoxic acid and those of 22 patients with miscellaneous diseases an average of 11.15 mg pyridoxic acid. In contrast, in 42 women with uncomplicated pregnancy at term the excretion averaged 8.84 mg and in 14 pregnant women with toxemia it was 8.53 mg pyridoxic acid. There was, therefore, no significant difference between toxemic and normal pregnant women, but there was a highly significant statistical difference ($P = < 0.001$) between the pregnant women and the normal control and miscellaneous patient groups. This would indicate a significantly greater retention of ingested vitamin B_6 in pregnancy as compared to the non-pregnant state.

Paper chromatographic methods have re-

cently disclosed that after the feeding of tryptophan, in addition to xanthurenic acid a variety of other metabolites are excreted by vitamin B_6-deficient but not by normal rats.[22] The urines of normal controls and of pregnant women were therefore examined for the presence of such metabolites following the ingestion of 10 grams of DL-tryptophan.[23]

The preparation of urine specimens according to Dalgliesh's original method[22] included precipitation with mercuric acetate, adsorption of the metabolites on deactivated charcoal, and elution of the charcoal by phenol. Unidimensional chromatograms from the concentrated eluates, when inspected under ultraviolet light, showed a number of clearly delineated fluorescent spots without significant background interference. These spots were identified as far as possible by their Rf values, specific color reaction, and with the aid of reference substances.

Normal control subjects after the ingestion of 10 grams of DL-tryptophan showed spots of which one was considered to be due to kynurenine, one to kynurenic and occasionally xanthurenic acid, and an inconsistent one to Nα-acetyl-kynurenine. In urine from pregnant women, six additional spots were seen. Of these, one was tentatively identified as 3-hydroxy-kynurenine, one as Nα-acetyl-3-hydroxy-kynurenine, and two as probably derivatives of 3-hydroxy-kynurenine. The abnormal excretion pattern is strikingly similar to that described in vitamin B_6-deficient rats,[22] but different from that reported in experimental riboflavin[24] and thiamine[25] deficiency. Moreover, following the administration of pyridoxine to pregnant women, the excretion pattern approximated that of normal controls.

In four women whose pregnancy was complicated by severe toxemia, the excretion pattern was similar to that seen in uncomplicated pregnancy. However, the observed spots appeared much more distinct and larger, paralleling the increased excretion of xanthurenic acid.

THE DIAZO REACTION

It is possible to demonstrate the occurrence of some tryptophan metabolites, including 3-hydroxy-kynurenine, in the urine of normal individuals on a regular diet without addition of tryptophan by means of column partition chromatography.[26] Increased amounts are excreted under abnormal conditions[26a]. The diazo reaction can be used for screening purposes, since it detects the presence of 3-hydroxy-kynurenine, as first demonstrated by Makino and co-workers in a case of advanced tuberculosis.[27] Later, Dalgliesh and Tekman[28] tested patients with various diseases and found a positive diazo reaction in some patients who had fever, regardless of the cause of the febrile state. In our own experiments a positive diazo reaction was found occasionally in patients with and without fever. Only in some of these cases could a positive diazo reaction be explained by the occurrence of 3-hydroxy-kynurenine.[29] The administration of pyridoxine led to a disappearance of the positive reaction in several of these cases. However, in view of the considerable spontaneous daily variation of the diazo reaction in the urine of these patients, no definite conclusion could be drawn. Makino and co-workers.[30] reported that the injection of pyridoxine to diazo-positive patients did not influence the excretion of 3-hydroxy-kynurenine in the urine.

Patients with various diseases whose urines gave a negative diazo reaction were then given the tryptophan load test. Some of these showed on chromatographic study an excretion pattern similar to that seen in pregnancy. Xanthurenic acid excretion in these cases was increased and varied between 8 and 85 mg (average: 40 mg). Among these were patients with hyperthyroidism, febrile disorders, and carcinoma. Repetition of the test following oral administration of vitamin B_6 led to a suppression of xanthurenic acid excretion, as well as to a normalization of the chromatographic excretion pattern.

DISCUSSION

Increased excretion of xanthurenic acid in patients with various diseases has been noted previously by us,[14] as well as in some patients with seborrheic dermatitis,[31] tuberculosis,[32] and diabetes.[33] In patients with diabetes, abnormal findings on paper chromatography

have also been reported by Kotake and Tani.[34]

Evidence for a disturbance of vitamin B_6 metabolism in all these instances is only circumstantial. However, the prompt correction of the metabolic defect by the administration of pyridoxine speaks in favor of a real deficiency or at least signifies an increased demand for the vitamin. Actual levels of vitamin B_6 have been measured in skin, blood, and urine and were not found different in pregnant and non-pregnant women.[35] However, normal tissue levels of vitamin B_6 are not necessarily indicative of "optimal" vitamin intake, sufficient, for example, to produce a maximal increase of body weight in growing rats.[36] Moreover, biochemical abnormalities, including increased xanthurenic acid excretion, appear before clinical symptoms become apparent in experimentally induced human deficiency.[37]

Whether an increased demand for vitamin B_6 in pregnancy and in some clinical conditions is caused by the same mechanism is not clear at present. Dalgliesh and Tekman[28] have suggested that the urinary excretion of tryptophan metabolites which occurs spontaneously in febrile states may be due to a relative insufficiency of the vitamin B_6-dependent enzyme system because of increased protein breakdown. In pregnancy, on the other hand, the growing fetus may compete with the mother for available vitamin B_6. This vitamin was shown to be essential for the growing chicken embryo by Cravens and Snell.[38] It is necessary for reproduction[39] and for normal fetal and infant development in the rat.[40] In view of the fact that supplementation of a normal diet with 10 mg of pyridoxine hydrochloride daily suppresses the abnormal excretion of xanthurenic acid, we have recommended such a supplement.[19]

The possible influence of a vitamin B_6 supplement on complications which occur in pregnancy was investigated in a group of 410 women. In a study conducted in co-operation with Dr. L. Graffeo, to be reported in detail elsewhere, a statistically significant decrease in the incidence of toxemia was observed in the treated as compared to a control group.[41] It is hoped that this favorable experience can be substantiated in a larger number of cases. Whether the administration of vitamin B_6 will influence the course of certain diseases, for example hyperthyroidism, in which biochemical tests indicate an increased demand for vitamin B_6, is as yet undetermined.

SUMMARY

Evidence for a biochemically demonstrable disturbance in the metabolism of vitamin B_6 during pregnancy is based on the following observations:

(1) A marked increase of xanthurenic acid excretion following a tryptophan load test.

(2) An abnormal excretion pattern of tryptophan metabolites on chromatographic analysis which is strikingly similar to that described in vitamin B_6-deficient rats.

(3) Immediate normalization of tryptophan metabolism following the administration of relatively small amounts of pyridoxine.

(4) An abnormal vitamin B_6 load test indicating a significantly larger retention of the administered vitamin in comparison to non-pregnant control subjects.

Some patients with hyperthyroidism, febrile diseases, and carcinoma exhibit a similar disturbance of tryptophan metabolism which responds to the administration of pyridoxine. The possible mechanism of this disturbance is discussed and certain therapeutic implications are pointed out.

ADDENDUM

Since this paper was submitted for publication, M. B. Glendening, A. M. Cohen, and E. W. Page (*Proc. Soc. Exper. Biol. & Med.* 90: 25, 1955) have found glutamic-aspartic transaminase activity of whole blood of pregnant subjects to be essentially the same as in non-pregnant controls. The activity was significantly increased by supplementation of the diet with 10 mg of pyridoxine hydrochloride daily. Transaminase activity of fetal blood was found to be twice as great as in maternal blood. The authors concluded that fetal tissues contain optimal quantities of vitamin B_6, whereas adults, both pregnant and non-pregnant, have suboptimal concentrations for maximal enzymatic activity. They concluded that their data were compatible with the view

that pyridoxine supplementation is desirable in human pregnancy.

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DISCUSSION

DR. E. E. SNELL (Austin, Texas): I should like to discuss this paper briefly and qualitatively from the standpoint of light that work with micro-organisms might throw upon the conclusions. The first conclusion that Dr. McHenry came to was that there is no failure of protein synthesis or maintenance in vitamin B_6-deficient rats. Work with micro-organisms, so far as it has gone, would confirm this conclusion completely. There is one experimental system that has been used extensively in the investigation of the role of vitamin B_6 in metabolism. This is the growth of an organism, *Streptococcus faecalis*, which is ordinarily dependent on vitamin B_6 in the medium for growth. However, under appropriate circumstances, which include the addition of D-alanine to the medium, this organism will grow without added vitamin B_6. Under the first set of conditions, where vitamin B_6 is required, one finds about 2 micrograms of vitamin B_6 per gram in cells when these are grown with the minimum amount of the vitamin that permits substantial growth. If one modifies the medium in such a way that growth occurs without vitamin B_6, one can get the same yield of cells and, therefore, the same amount of protein synthesis. However, the vitamin B_6 content of the cells harvested under these conditions is in the neighborhood of 0.03 microgram per gram. This figure is *far* lower than the amount of vitamin B_6 that can be obtained in any animal cell under any condition of vitamin restriction. Since such cells synthesize protein readily (when a complete array of amino acids is provided), it would be unexpected to find a limited ability for protein synthesis in the much less rigorous vitamin B_6 deficiency that can be induced in animal cells by dietary restriction of vitamin B_6 intake.

This last statement is true, however, only when each of the amino acids necessary for protein synthesis is furnished; that is, given a complete assortment of amino acids, vitamin

B_6 is not required to put them together into protein. However, if we omit any one of the amino acids that goes into the makeup of protein in *S. faecalis*, then this organism will not grow *unless* vitamin B_6 is supplied. Furthermore, the amount of vitamin B_6 that one must supply varies considerably with the amino acid that is omitted. We may conclude that, while a requirement for vitamin B_6 for protein synthesis from preformed amino acids has not been demonstrated, the role of this vitamin in the synthesis of each of the amino acids has been demonstrated. Furthermore, the vitamin B_6 requirement for individual synthetic processes in these cells varies markedly. *S. faecalis* does not degrade amino acids supplied in excess to satisfy its energy requirements, as do animals. Degradation of amino acids also requires vitamin B_6, and hence in animals (unlike *S. faecalis*), the vitamin B_6 requirement is increased when proteins are fed in excess of the minimum amounts required. It would be expected from the bacterial experiments that the requirement of animals for vitamin B_6 would also be enhanced under conditions such that synthesis of "non-essential" amino acids is required; this possibility has not been tested experimentally as yet.

In connection with the decrease in activity of pyridoxal phosphate-dependent enzymes with time on a vitamin-restricted diet, it is important to recognize that every such enzyme may behave independently. At least twenty-odd enzymatic processes within the animal body are dependent upon pyridoxal phosphate. In the vitamin-deficient animal, each of these enzymes will be competing for a limited and insufficient supply of pyridoxal phosphate. Under these circumstances, those apoenzymes that combine relatively loosely with the coenzyme might decrease rapidly in activity with time, whereas those apoenzymes that combine firmly with the coenzyme might decrease in activity only very slowly, if at all, on the deficient regimen. Dr. McHenry's results suggest that the glutamic-aspartic transaminase is an enzyme of this latter type; results of others with cysteine desulfhydrase and cysteine sulfinic acid decarboxylase (which fall to very low levels within a few days after withdrawal of the vitamin from the diet) suggest that these are enzymes of the former type. That is, if A, B, C, D, etc. represent pyridoxal phosphate-dependent apoenzymes,

$$\text{A} + \text{pyridoxal phosphate} \overset{K_1}{\rightleftharpoons} \text{enzyme A}$$
$$\text{B} + \text{pyridoxal phosphate} \overset{K_2}{\rightleftharpoons} \text{enzyme B}$$
$$\text{C} + \text{pyridoxal phosphate} \overset{K_3}{\rightleftharpoons} \text{enzyme C}$$
$$\text{D} + \text{pyridoxal phosphate} \overset{K_4}{\rightleftharpoons} \text{enzyme D}$$
$$\text{etc.}$$

then $K_1 \neq K_2 \neq K_3 \neq K_4$ and the enzymes may decrease in activity at very different rates following withdrawal of vitamin B_6 from the ration. It is important to note that even if no decrease whatever in a single enzyme were observed following long periods on the vitamin-deficient ration, this would still not constitute evidence against the possibility that the vitamin was essential for activity of that enzyme, for even in highly deficient animals the total vitamin B_6 concentration in the carcass is rarely reduced below one-third of that in controls.

A related point concerns the activity of vitamin antagonists in producing vitamin deficiency symptoms. All investigators agree that these compounds (e.g. 4-desoxypyridoxine or its 5-phosphoric acid ester) act by competing with pyridoxal phosphate for the apoenzymes A, B, C, D, etc., or in displacing pyridoxal phosphate from the corresponding enzymes. Now, there need be no correlation between values for K_1, K_2 etc. (governing the association of apoenzymes and coenzyme), with values of K_A, K_B etc. (governing the association of A, B, etc. with the vitamin analogue). This means that a compound such as desoxypyridoxine, for example, may act exclusively as a vitamin B_6 antagonist, and yet produce a different set of deficiency symptoms from that produced by dietary restriction of vitamin B_6, because a different set of vitamin B_6-dependent enzymes is affected. Examples of this type of action are already in the literature. Thus, although it is highly important to learn the actual enzymatic disabilities produced by vitamin restriction, data of the type Dr. McHenry is obtaining, it is also important to realize the limitations of this type of approach in delineating the primary

functions of a particular vitamin in metabolism.

DR. JANET L. STONE (Nashville, Tenn.); While we are discussing the role of vitamin B_6 and transaminase, I thought I would like to point out that the measurement of serum level of glutamic-oxalacetic transaminase activity has received rather wide clinical application recently. About a year ago Dr. LaDue and his group, including Dr. Wroblewski and Dr. Karmen, announced that the transaminase activity—by that I mean specifically the glutamic-oxalacetic transaminase activity—rose 2 to 20 times normal in patients suffering from myocardial infarction. The level of this enzyme first rose about 12 hours after the onset of pain and it continued to rise and reached a peak about 24 hours after the onset of pain and returned to normal in two or three days following the attack. At this time they suggested that this test might be helpful in diagnosis of myocardial infarction. We became interested in this problem at Thayer Hospital when we heard Dr. LaDue's announcement, and of course we wondered what was the source of the increased enzyme activity in the serum. We produced experimental myocardial infarction in dogs by ligation of the coronary artery. When serum samples from these dogs were examined, it was found that they reproduced the pattern seen in humans after myocardial infarction. There was a striking increase of 2 to 20 times normal in the level of enzyme activity in the serum, and it again returned to a normal level several days after the operation. Necrotic cardiac tissue samples from the infarcted area showed decreases in activity of transaminase, and these tissue decreases were proportional to the increases seen in the serum. The decreases in tissue enzyme activity were also proportional to the estimated size of the infarct.

At the same time that we were doing these experiments at Thayer, Dr. LaDue's group and another group in California, composed of Agress, Clark, and others, were performing similar experiments on dogs. As a result of our findings and similar findings reported by these groups, it seems fairly well established now that the source of the increased transaminase level in the serum is the necrotic cardiac tissue. I don't mean to imply that only necrotic *cardiac* tissue can contribute transaminase to serum. There is also an increase in serum transaminase activity following extensive injury to skeletal muscle, or in patients suffering from liver disease involving hepatic cell damage. Apparently any necrotic cell releases transaminase, and this may be reflected by an elevation of the serum level. However, along with the clinical picture, the estimation of serum transaminase activity is a valuable aid in the diagnosis of myocardial infarction. It has now been applied in a large series of patients; and I might mention that the method for measuring serum transaminase as proposed and worked out by Drs. Wroblewski, Karmen, and LaDue is very simple. It is quick, easily performed, and the only special apparatus required is the spectrometer.

DR. W. J. MCGANITY (Nashville, Tenn.): I would like to make a few comments referable to Dr. Wachstein's presentation of his work with obstetrical patients. The problem of specificity which was raised this afternoon must be modified further in the pregnant subject. In this patient, we must deal not only with the specificity of the tryptophan load test, but also with the influence of pregnancy *per se*, as reflected by the apparent increased response to the DL-tryptophan load during gestation, and then declining in the postpartum period. This pattern is similar to our own results during gestation with N′-methylnicotinamide excretion following a load test with niacin.

Is xanthurenic acid an abnormal excretion product for the pregnant person? I don't believe we can answer the question at present. Certainly normal individuals with no evidence of toxemia exhibit this response to the tryptophan load. Is this a pathologic or physiologic variant? Toxemia is a disease of the second half of pregnancy. Any statistical comparisons between the "normal" and "abnormal" response should be restricted then to that phase of gestation (24–40 weeks gestation). One further point on specificity is the relationship to 4-pyridoxic excretion. All of us will

agree that it may reflect an increased retention of vitamin B_6 within body tissues of the human adult or other test animals. However, does it reflect increased utilization of vitamin B_6 by the subject? There is some evidence to indicate that it doesn't, and particularly during pregnancy. Early in pregnancy, the pregnant woman develops a positive nitrogen balance. There is a decline in blood urea levels[1] which does not support the thesis that there is an increased demand for or utilization of pyridoxine in either deamination or transamination within the body.

About two years ago we initiated a study which attempted to superimpose pregnancy on female rats which were fed pyridoxine-free diets—diets which will result in hypertension.[2] We hoped that we might produce an entity in the experimental animal that would approximate toxemia as seen in the obstetric patient. Under conditions used to date, we have been unsuccessful. However, several observations indicate a need for pyridoxine in normal estrus, conception, fertilization, and gestation of the rat. During the initial phase of the study we were unable to produce estrus on our deficient diet, so pregnancy was impossible. In an arbitrary manner we have added minimal amounts of oral pyridoxine to our regimen. Group I received 10 μg per day, Group II 5 μg per day, Group III 2 μg per day, and Group IV continued without supplemental vitamin B_6.

Performance in these groups[3] has varied, as reflected in the ability of the animal to conceive, the litter size, litter weight, lactational performance, the stillbirth and neonatal death rate of the pups, and the occurrence of convulsive seizures in the offspring. This latter phenomenon occurred among pups born to mother rats in Groups II and III. About the seventh day of life the young developed a spasticity and an ataxia of gait, followed on the tenth day by spontaneous generalized convulsive seizures. By the eleventh or twelfth day they became anoxic and died. So far we have been unable to control or prevent these convulsions with the administrations of large amounts of pyridoxine. Studies at present in progress are designed to determine minimal and optimal amounts of vitamin B_6 required by the rat for normal obstetric and pediatric performance.

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Dr. B. T. Towery (Nashville, Tenn.): The observations of both Dr. Wachstein and Dr. McGanity are very important to the neurologist, because he is confronted with a large group of patients in whom seizures come on some time after birth with no apparent cause in terms of birth injury, infection, and so forth. If Dr. Wachstein's data suggest and are proved to show a relative deficiency of pyridoxine in at least some women during pregnancy, this may be one of the factors which goes on to produce seizures and several types of cerebral palsy in the children born from such mothers. The studies that Dr. McGanity has shown us are consistent with this possibility. And, if I may have Dr. Coursin's permission to quote some of his unpublished studies, he has the impression that some of his patients represent the analogy in man to Dr. McGanity's rats, in that they have developed seizures after what was apparently pyridoxine deficiency in infancy and are now no longer responsive to pyridoxine therapy. I think this is a very important development which we all should watch and work on.

The Metabolism of Vitamin B_6 in Human Beings

By RICHARD W. VILTER, M.D.*

MUCH HAS been written and spoken about vitamin B_6 metabolism in the rat, the monkey, the dog, and the pig. By analogy, we can surmise a great deal about the functions of this vitamin in man, whose welfare is our principal concern. Yet, a function that is clearly established in one or more animals need not be important or even occur in human metabolism. Therefore, I shall sketch as briefly as possible what is known about vitamin B_6 in human beings, drawing from work done in many laboratories, including our own. This review will point out the many gaps in our knowledge, because the gaps loom larger than the solid facts.

The vitamin B_6 complex, including pyridoxine, pyridoxal, and pyridoxamine, is widely distributed in many human foodstuffs. There are, however, insufficient data to warrant publication of a table on the vitamin B_6 content of a variety of common foods. Analyses of our regular diet at the Cincinnati General Hospital, as it is presented to the patient, indicate a daily intake of between 1 and 1.5 mg, proving that previous figures, based on rough calculations, were not much in error. Estimates of the human daily requirement for vitamin B_6 range between 1 and 2 mg. These are based on analogy with rat requirements[1] and on studies of vitamin B_6 deficiency in human beings induced by the antagonist, desoxypyridoxine.[2] It is clear that the estimated daily requirement and the amount of the vitamin supplied by a good hospital diet agree very nicely.

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ABSORPTION

Vitamin B_6 is absorbed rapidly and completely by human beings, though that part of the intestine through which maximum absorption occurs is not known. Pyridoxal is absorbed more rapidly and completely than its two relatives.[3] Organisms in the gastrointestinal tract manufacture a considerable amount of vitamin B_6. Although rats make use of this source of supply,[4] it is doubtful that humans do. Several short studies by Sarett[5] and unpublished works from our Laboratory suggest that little, if any, vitamin B_6 is obtained by human beings from this source. Reports from Italy[6] of results quite opposite to these must be criticized because antibiotics used to destroy intestinal organisms were easily absorbed and excreted in the urine and stool. They undoubtedly had a profound inhibitory effect on the micro-organism, *Streptococcus faecalis*, used to measure vitamin B_6. Though about 0.8 mg of vitamin B_6 is usually excreted in the stool each day, this is derived primarily from dead and living micro-organisms, and is probably not at any time available to the human host.

EXCRETION

Vitamin B_6 is excreted in the urine in several forms. Pyridoxal and pyridoxamine are the principal active vitamins. There is almost no pyridoxine.[3] The total amount of these vitamins as measured by the yeast, *Sacch. carlsbergiensis*, varies between 0.2 mg and 0.3 mg. Pyridoxic acid is the chief excretory product, and this substance can be measured only by a photofluorometric method. Since this method measures fluorescent substances other than pyridoxic acid,[7] it is probably in error by 50 per cent or more when subjects are not receiving vitamin B_6 supplements or test

doses. The usual pyridoxic acid level in persons on a normal diet is 3.5 to 4 mg per day, but this is obviously much too high. The true value must be closer to 1.5 mg. Since the non-specific fluorescent materials are rather constant, the test is valuable for comparative purposes or to measure responses to a load test of pyridoxal. However, after such a load test in normal persons only 70 per cent of the ingested vitamin can be recovered[3] (in the case of pyridoxine, 45 per cent, and after feeding pyridoxamine, 31 per cent). These discrepancies suggest that other unrecognized metabolites of vitamin B_6 may appear in the urine, also.

TABLE I

Vitamin B_6 Metabolism in Adult Human Beings

	Vitamin B_6 in mg/day		
	Normal	Deficient*	Deficient + 15 Mg Pyridoxine*
Average Daily intake	1.5	0.75	15.75
Average daily output			
Urine vitamin B_6			
Pyridoxal†	0.05		
Pyridoxamine†	0.20		
Pyridoxine†	0.00		
Total	0.25	0.09	1.00
4-Pyridoxic acid	3.50	2.4	8.87
Stool vitamin B_6	.80	0.71	0.81

* Data from Linkswiler.[9]

† Data from Rabinowitz and Snell.[3]

Tables I and II demonstrate these various relationships and indicate that we cannot do satisfactory balance studies until we know how much vitamin B_6 may be supplied by intestinal organisms, how much true pyridoxic acid appears in the urine under normal conditions, and what additional urinary excretory products of vitamin B_6 there may be. Møller,[8] and Linkswiler and Reynolds[9] have done as much as is now possible to establish vitamin B_6 outputs in adult and infant subjects on various dietary intakes.

BLOOD LEVELS

Blood levels of vitamin B_6 and of its coenzyme, pyridoxal phosphate, in fasting human subjects, are so low that methods of assay cannot give accurate results. Vitamin B_6 averages 5 μg/100 ml; pyridoxal phosphate, one-tenth this amount.[10] These compounds are about equally distributed between plasma and cells. Tissue levels have not been determined sufficiently often in human beings to warrant a quotation of values, though the level for normal skin appears to be about 1 μg/gram dry weight.[11]

TABLE II

Vitamin B_6 Metabolism in Infants*

	Vitamin B_6 in mg/day	
Average daily intake	0.70	1.68
Average daily output		
Urine vitamin B_6		
Pyridoxal	0.08	0.16
Pyridoxamine	0.05	0.13
Pyridoxine	0.00	0.00
Total	0.13	0.29
4-Pyridoxic acid	0.55	1.21
Stool vitamin B_6	0.15	0.31

* Data from Møller.[8]

CLINICAL FUNCTIONS

The functions of vitamin B_6 in the human body can be assessed only through studies of the derangements that occur during spontaneous or induced deficiency states and of restoration of function during the period of recovery. Only in infants has dietary restriction of vitamin B_6 induced clinical as well as biochemical abnormalities. In 1950, Snyderman, Holt, Carretero, and Jacobs[12] studied two hydrocephalic infants maintained on a synthetic diet devoid of vitamin B_6. After 76 days, one of these infants developed convulsions, and after 130 days the other developed anemia. Both excreted large amounts of xanthurenic acid in the urine after a load test of tryptophan and failed to excrete a normal amount of N^1-methylnicotinamide. All of these abnormalities disappeared after pyridoxine was administered, the N^1-methylnicotinamide excretion being the last to return to normal.

We have heard this morning how vitamin B_6 deficiency was induced inadvertently when a commercial liquid milk product was processed in such a way that the vitamin B_6 content was reduced to about 60 μg/liter, and the fatty acid content changed at the same time. Apparently, as a result of either or both of these maneuvers, convulsive seizures occurred in

many infants fed this product. In most of the infants, dietary improvement was sufficient to relieve the convulsions. In one infant, an intramuscular dose of 100 mg pyridoxine relieved the convulsions and restored the electroencephalographic pattern to normal in five minutes.[13]

These studies in infants indicate that deficiency of vitamin B_6 upsets the complex processes of cerebral metabolism and causes convulsions. The suggestion that this metabolic abnormality occurs in the Krebs energy cycle is quite plausible, since vitamin B_6 is known to function in glutamic acid—α-ketoglutaric acid interconversion. In addition, vitamin B_6 influences blood formation, presumably through the formation of the pyrrole ring. It is involved also in the formation of N^1-methylnicotinamide from tryptophan. One result of the block in tryptophan metabolism caused by vitamin B_6 deficiency is the excessive excretion of xanthurenic acid.

EXPERIMENTAL PYRIDOXINE DEFICIENCY IN MAN

Vitamin B_6 deficiency states have not been induced in adults by dietary means alone. Hawkins and Barsky[14] did not observe any pathognomonic symptoms or signs after their subject had been on a synthetic vitamin B_6-deficient regimen for 54 days. Greenberg and Rinehart[15] observed an increase in the excretion of xanthurenic acid in the urine of their subjects who had been on a similar regimen for three weeks, but no other abnormalities were noted. We[16] have maintained a patient with pellagra for nine weeks on our B-complex-deficient diet supplemented with niacin, thiamine, and riboflavin. The premise that vitamin B_6 deficiency might be precipitated by treating such a deficient patient with the three main B-complex vitamins did not prove to be true. Though the urine vitamin B_6 levels remained low (80–100 μg/24 hours), glossitis and dermatitis cleared rapidly, and the peripheral neuritis slowly improved. No new lesions developed. Xanthurenic acid levels were only moderately elevated.

The situation was different, however, when 50 subjects were given a metabolic antagonist of vitamin B_6, 4-desoxypyridoxine.[1] Some subjects were maintained on a vitamin B_6-deficient diet, and others on a normal hospital menu. The deficiency state developed more rapidly in those on the deficient regime, but occurred in both groups, and could be reversed completely by pyridoxine or its relatives, and, partially, by the essential fatty acid, linoleic acid. Many of the patients became irritable, depressed, and sometimes somnolent. Seborrheic dermatitis developed in the nasolabial folds, in the eyebrows, and around the eyes in 29 individuals. It spread over the face, forehead, and chin, up into the hair, and down the neck. In several persons it involved the perineal region also. Intertrigo developed under the breasts and in the groin in several women, and, occasionally, a hyperpigmented scaling pellagrous-like dermatitis developed on the arms and legs. Cheilosis, angular stomatitis, and conjunctivitis developed in six. Glossitis was noted in 14, and peripheral neuritis was a prominent feature in three. Lymphopenia occurred regularly, but anemia could not be related to the vitamin B_6 deficiency state. After tryptophan administration, large amounts of xanthurenic acid appeared in the urine. Blood urea nitrogen levels were elevated, and, after the administration of alanine, remained so for longer than 12 hours. When infections occurred, usually in the form of cystitis or pyelonephritis, there was a poor response to antibiotics which were effective *in vitro* against the offending micro-organisms. After vitamin B_6 was administered, the infections responded well. All of these abnormalities were relieved by the members of the vitamin B_6 group, but were not influenced by thiamine, niacin, or riboflavin. Linoleic acid relieved the skin lesions, but had no effect on the other clinical or laboratory features. This study indicates that vitamin B_6 deficiency in human beings is not very different from the syndrome observed in laboratory animals.

Though we recognize that vitamin antagonists often do not reproduce the complete deficiency state, but rather may alter one enzyme system maximally while leaving another entirely unaffected, the clinical picture just described is the closest that investigators have

come to vitamin B_6 deficiency in the adult human being. Skin, mucous membrane, and nerve tissue are affected. Lymphocytes are decreased and infections respond poorly, suggesting a reticuloendothelial abnormality which interferes with antibody production. Tryptophan metabolism is abnormal and blood urea nitrogen is elevated. The skin response to linoleic acid suggests a relationship to essential fatty acid metabolism. It is well known that all these biochemical areas require vitamin B_6 for activation.

If spontaneous vitamin B_6 deficiency states occur in adult human beings due to dietary insufficiency, they are very rare. Spies and his co-workers[17] have reported a syndrome characterized principally by muscle weakness which occurred in pellagrins and responded to vitamin B_6. Hammouda and Sidky[18] reported similar findings in Egyptian pellagrins, and Smith and Martin[19] found that cheilosis, which was unresponsive to riboflavin, could be cleared by pyridoxine. Biochemical proofs that these were, indeed, pyridoxine deficiency states were unavailable at the time of these reports.

STUDIES WITH INH

Several other studies have indicated metabolic activity of vitamin B_6 in human beings. One of my associates, J. Park Biehl, and I were able to prevent the neuritis which occurred in patients who received large doses of isonicotinic acid hydrazide (INH) by giving pyridoxine concurrently.[20] From 2 to 3 per cent of patients receiving conventional doses of INH (2–3 mg/kg) developed neuritis, whereas 40 per cent of patients receiving 20 mg/kg develop this abnormality. Fifty mg of pyridoxine daily was sufficient for prophylaxis. When this work was begun, we were aware that INH inactivated certain decarboxylase enzyme systems which required pyridoxal phosphate as coenzyme, and that peripheral neuritis occurred in our patients treated with 4-desoxypyridoxine. Increasingly large doses of INH increased the amount of pyridoxal, but not pyridoxic acid found in the urine; xanthurenic acid excretion after tryptophan also increased, but in an irregular fashion. For these reasons, we proposed that INH may couple with pyridoxal to form a pyridoxal-INH hydrazone, thus inactivating enzyme systems dependent on pyridoxal and causing large amounts of a hydrolyzable vitamin B_6 compound to appear in the urine. Direct antagonism might also occur between INH and pyridoxal phosphate at the apoenzyme level. These studies indicate that new drugs may block certain aspects of vitamin metabolism. In the case of INH, only nerve tissue and tryptophan metabolism seem to be affected. Other manifestations of desoxypyridoxine-induced vitamin B_6 deficiency have not been observed. If the enzyme systems inactivated by INH can be discovered, a direct chemical link between vitamin B_6 and nervous system function will be established.

PREGNANCY

For many years pyridoxine has been used empirically in women with nausea of pregnancy. Only recently a physiologic abnormality involving vitamin B_6 has been demonstrated in the pregnant woman. Dr. Wachstein has reported this afternoon that pregnant women in the second and third trimester, given a test dose of tryptophan, excrete abnormally large amounts of xanthurenic acid in the urine when compared with non-pregnant women. This abnormality is quickly relieved by pyridoxine. There is also a small but statistically significant difference between the amount of 4-pyridoxic acid that appears in the urine of pregnant and non-pregnant women after a load test with pyridoxal.[21] The suggestion has been made that pregnant women are deficient in vitamin B_6, due to dietary inadequacies, the demands of the fetus, and vomiting. However, there are no reports of clinical symptoms and signs in pregnant women ascribable to vitamin B_6 deficiency. It has seemed to us that the pregnant woman may have basic abnormalities in tryptophan metabolism and related processes which can be overcome by an intake of pyridoxine that is higher than usual. In the strict sense, this would not constitute a vitamin B_6 deficiency, but rather an increased demand at one or more biochemical levels. Excretion of pyridoxic acid after a pyridoxal

load might be reduced by this demand. Dr. Friedman[22] has measured vitamin B_6 levels in the blood, urine, and skin of pregnant women who have the tryptophan abnormality. Blood levels, though unsatisfactory, are in the normal range. Urine and tissue levels are normal or high. Therefore, it seems likely that a supposedly physiologic state such as pregnancy causes metabolic changes which increase the need for vitamin B_6, at least in so far as tryptophan metabolism is concerned. Dr. McHenry's group[23] has published evidence that urea nitrogen metabolism after a test dose of alanine is affected in a similar fashion.

SEBORRHEIC DERMATITIS

Another suggestion that vitamin B_6 needs may be increased by unknown circumstances comes from the observation that many patients with the sicca type of seborrheic dermatitis will respond to local application of pyridoxine, but not to oral or parenteral administration of this vitamin.[24] Though this observation has not been confirmed, Dr. Friedman in my Laboratory has demonstrated that much more pyridoxine can be found in the skin of persons receiving the vitamin locally, compared with those who have been treated parenterally or orally. If a metabolic abnormality exists in the skin of such patients, the defect probably lies at the level of the essential fatty acids.

COMMENT

In these various ways, the mysteries of vitamin B_6 metabolism are being probed by numerous investigators. The results are never as definite or clear-cut as one can obtain from animal experiments.

Often implications only can be drawn concerning metabolic relationships. These studies show, however, that vitamin B_6 is a very important substance in the diet of adult human beings, that deranged vitamin B_6 metabolism induces lesions in skin, mucous membranes, nerve tissue, and lymphatic system, and that the metabolism of tryptophan, protein, carbohydrate, and fatty acids may be affected adversely by this deficiency. In addition, some of these studies suggest that specific metabolic abnormalities may increase the need for vitamin B_6, and that drugs may cause toxic manifestations by interfering with the function of this vitamin. These are physiologic concepts which, if true, transcend vitamin B_6, for they may apply to the physiology of all essential nutrients.

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DISCUSSION

DR. W. H. SEBRELL, JR. (New York, N. Y.): The human metabolism of vitamin B_6 is a most fascinating subject to discuss because it is so broad and has so many possibilities that it leads to a number of interesting thoughts, although one may not be able to draw any definite conclusions. First, the question of whether there is any human need for, or human metabolism of, vitamin B_6 seems to be pretty well answered with a great deal of information, some of it indirect and some of it direct. I suppose the most direct evidence comes from the observations of Dr. Holt and his associates on the two children that were reported in 1951, one of the children having developed convulsive seizures on the 76th day of vitamin B_6 depletion, which were relieved by the administration of vitamin B_6. This was followed by the accidental and unfortunate natural occurrence of the condition which was reported by Dr. Coursin this morning. So it seems pretty well established, both from these data and from the material covered by Dr. Vilter, that there certainly is some necessary human metabolism of vitamin B_6.

If this is so, then why don't we have more definite evidence on the human needs for vitamin B_6? This vitamin differs from several others. In the case of ascorbic acid, niacin, and thiamine deficiency, there is a widely occurring human disease with which one could demonstrate the necessity of the vitamin. This doesn't seem to be the case with vitamin B_6, probably because of its widespread availability. Now if we assume, as we do with the other vitamins, that animal lesions resulting from deficiency bear some resemblance to what happens in the human, and that the metabolic processes are probably similar if not identical—and that the lesions as they occur are probably, first, a biochemical defect, secondly, an accumulation of an abnormal metabolite or disappearance of an essential metabolite, and, finally, the occurrence of cellular alterations (such as Dr. Vilter has demonstrated by the use of the antagonists)—then we may get evidence for the need of the compound without having any clearly defined clinical syndrome on which you can put your finger. This is indicated by the work of Dr. Wachstein and the others who have shown the increased excretion of xanthurenic acid. If we assume that increased xanthurenic acid is a manifestation of a biochemical defect, then this perhaps represents one of the early stages of deficiency or inadequate supply of vitamin B_6, although it may be only in one particular system, that involved in the degradation of tryptophan.

If I understood Dr. McHenry rightly, he wasn't questioning the basic observation that an increased xanthurenic acid excretion occurs with vitamin B_6 deficiency, but was expressing

doubt concerning the specificity of this reaction, and I think rightly so, because certainly a complex reaction such as the degradation of tryptophan to niacin could possibly be interrupted at many different points and one could get an increased excretion of xanthurenic acid by some mechanism other than a direct deficiency of vitamin B_6. Nevertheless, it does seem to be closely enough related to be a practical observation of considerable importance. Incidentally, this original observation, you will remember, goes back to animal work which Elvehjem and his group did when they showed that in spite of large amounts of tryptophan in the animal's diet, niacin was still required if there was no vitamin B_6 in the diet, indicating that the pathway from tryptophan to niacin was blocked unless vitamin B_6 was present, and Lepkovsky and his group at California demonstrated the increased xanthurenic acid excretion.

The nausea and vomiting of pregnancy could well be related to the increased demand on the pregnant woman for protein formation. If pyridoxine is essential in protein metabolism, the pregnant woman with an increased demand for manufacturing new protein certainly should logically have a greater requirement for vitamin B_6 than the normal adult. This would be in accord with our notions about the requirements for dietary essentials varying very widely under different conditions of stress. There is one clinical observation that hasn't been mentioned at this meeting, and that is the clinical use of vitamin B_6 in radiation sickness. Nausea and vomiting is one of the manifestations of this condition, and I think it was in 1943 that Maxwell and his associates first made the clinical observation on the beneficial effect of vitamin B_6 which has since been repeatedly observed in many clinics. Now, the underlying mechanism of what happens in radiation sickness is totally unknown, but there is the observation that the administration of the vitamin has some beneficial effect on the clinical symptoms. The symptoms could be related to some sort of interference with protein metabolism caused by the heavy radiation. I have never seen anything in the literature on xanthurenic acid excretion following a tryptophan load test in radiation sickness. This is such a simple clinical observation I suppose somebody must have done it, but it has escaped my notice.

To go back to the convulsive seizures in infants, the effect on the nervous system has been widely discussed here. I understand Dr. Tower talked about glutamic acid this morning, and if pyridoxine is related not only to transamination but to decarboxylation of glutamic acid, and if glutamic acid is present in considerable amounts in the central nervous system, it seems quite reasonable to suppose that any disturbance in vitamin B_6 metabolism as it might affect glutamic acid could be related to changes in the central nervous system.

Finally, another field of interest is embraced by the observations reported to you this morning by Dr. Rinehart and Dr. Emerson concerning the production of atherosclerosis in the monkey. In the present state of our knowledge, I wouldn't even presume to guess as to the mechanism of the production of atherosclerosis in man, but it may be related in some way to fat metabolism. Witten and Holman in 1952 noted the role of vitamin B_6 in the formation of arachidonic and hepanoic acid from linoleic and linolenic acids and there is, of course, a possibility that this could play some role in the mechanism of the production of atherosclerosis. Dr. Vilter mentioned acne, which again could possibly be related to fat metabolism.

DR. D. TOWER (Bethesda, Md.): Dr. Vilter pointed out and Dr. Sebrell has further emphasized the idea that one of the problems which we have is that we do not know enough about the metabolism of pyridoxine and its related compounds in animals or man. Since so many of the symptoms of deficiency involve the central nervous system, it becomes important to know how much pyridoxine gets across into the central nervous system compartment normally and after treatment. Dr. Boxer has been kind enough to tell me that in cats and rats about 25 to 50 per cent of the pyridoxal phosphate level in the blood is found in cerebrospinal fluid.

On one of the patients which I spoke about

this morning Dr. Boxer kindly carried out assays for the various vitamin B_6 compounds in the spinal fluid. This patient was on a dose of 50 milligrams of vitamin B_6-hydrochloride five times daily and had been on this dosage for six days at the time the sample was taken. The sample was taken in the morning after the first dose given 11 hours previously. He received 50 mg by mouth and $2^1/_2$ hours later the assay in the cerebrospinal fluid, in micrograms per 100 milliliters, was as follows: pyridoxal phosphate, 4, pyridoxal, 3, pyridoxine itself, 13, and pyridoxamine, none. Dr. Boxer felt that these levels, particularly of pyridoxal phosphate, were higher than would be expected from just a single dose of this magnitude, so that perhaps some had carried over from previous doses. It indicates to me that adequate quantities are getting through into the cerebrospinal fluid. In other words, the studies on cats and rats are borne out in man in that there is no appreciable barrier to the crossing of these substances into the cerebrospinal fluid, and that a considerable proportion of the total is in the coenzyme form.

Now I present this not just to have something to say, but to emphasize the necessity of having more studies of this sort, and preferably serial studies, so that we can know what we are doing when we give vitamin B_6 to these people. This is the type of information we are going to need in order to interpret what we are accomplishing in our patients and in our animals.

There is one other thing I would like to say in relation to the last paper, and that is about the creatinuria which occurs. Dr. Vilter, I believe, previously brought the question of energy metabolism into this problem. Now, it has been shown recently that in muscular dystrophy and similar conditions there is a marked decrease in the glycolytic activity of such muscle, and in particular of aldolase, which is an important enzyme in the early stages of the utilization of carbohydrate for muscle energy metabolism. It is likely, on the basis of theoretical and experimental considerations, that the creatinuria which occurs as a result of muscular dystrophy and other muscle dysfunctions is due to liberation of creatine from creatine phosphate, because of the inability of the muscles to metabolize carbohydrate into high-energy phosphate in the normal fashion. This may explain the creatinuria in this case. It is quite interesting that the administration of alpha-tocopherol and possibly vitamin B_6 helps in correcting the creatinuria. I do not quite know what the explanation for this is, but I think it should be followed up.

Nutritional Muscular Dystrophy in Monkeys Receiving a Diet Deficient in Both Vitamins B_6 and E

By PAUL L. DAY, PH.D.* AND JAMES S. DINNING, PH.D.†

SEVERAL years ago we observed unmistakable signs of vitamin E deficiency in monkeys receiving a diet containing, among other ingredients, polished rice and whole wheat. However, the diet contained a small amount of tocopherols and was therefore not well suited to the study of vitamin E deficiency. On this diet, muscular weakness and the chemical evidences of vitamin E deficiency developed only after two or three years.[2]

Recently we[3–5] have obtained evidence on lower animals suggesting a metabolic interrelationship between vitamin B_6 and vitamin E. Thus, when weanling albino rats were given a diet deficient in both vitamins B_6 and E they developed a leukocytosis characterized by greatly increased peripheral neutrophil counts. Supplementation of the diet with either of these vitamins prevented the increase. The vitamin B_6-deficient rats exhibited a slight lymphopenia which was not affected by vitamin E. None of the rats developed an anemia during the period of the experiment. Some of the data are shown in Table I.

In such experiments the rats deficient in vitamin E alone grew nearly as well as those receiving both vitamins. On the other hand, rats deficient in vitamin B_6 grew almost as poorly as those deficient in both vitamins.[4] Both vitamins, however, influenced the excretion of creatine and allantoin. The deficient (basal) group excreted large amounts of creatine, and *either* vitamin prevented this creatinuria. Similarly, the rats deficient in both vitamins excreted increased amounts of allantoin, and this was prevented by either vitamin B_6 or vitamin E.

TABLE I

Effects of Vitamins E and B_6 on Peripheral Leukocyte Counts in Rats
(Cells in thousands per microliter)

Diet	Total leukocytes	Lymphocytes	Monocytes	Neutrophils	Eosinophils
Basal (deficient)	18.4	6.9	0.6	10.5	0.4
" + E	8.5	5.7	0.1	2.4	0.1
" + B_6	10.0	7.2	0.4	2.2	0.2
" + E + B_6	10.8	8.8	0.4	1.5	0.1

In view of this apparent interrelationship between vitamin B_6 and vitamin E in the metabolism of the rat, it seemed possible that such a double deficiency might accelerate the production of vitamin E deficiency in the monkey. Consequently, we have subjected a number of young rhesus monkeys to a diet of purified materials which is deficient in both vitamins.

MATERIALS AND METHODS

The composition of the diet is shown in Table II.

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TABLE II

Composition of Diet Deficient in Vitamin E and B_6

(Daily allowance per monkey)

	grams		*mg*
Casein, vitamin-free	18	Riboflavin	0.5
Lard	8	Calcium pantothenate	2.0
Salt mixture	4	Nicotinic acid	2.0
Choline chloride	0.1	Menadione	0.44
Inositol	0.1	Folic acid	0.5
Corn starch	46.2	Thiamine chloride	0.5
Sucrose	21.6	Ascorbic acid	20.0
Baking powder	1.5		
Cod liver oil	3.0		

The casein furnishes essential amino acids, lard and cod liver oil supply essential fatty acids, the salt mixture supplies all of the required inorganic elements, and the starch and sucrose yield energy. All of the vitamins believed to be required by the monkey are included, except vitamins B_6 and E. The diet ingredients, except the cod liver oil, thiamine chloride, and ascorbic acid, are mixed together with enough water to make a thick batter and then cooked in a moderate oven in the form of a crisp wafer. The baking powder gives a light texture to the wafers. The cod liver oil, thiamine chloride, and ascorbic acid are placed on the wafers just before feeding. The monkeys pick up the wafers and eat them greedily.

The animals are housed in steel metabolism cages and given this diet and water once daily. The cages are equipped with funnel-shaped stainless steel trays for the collection of urine. The 24-hour urine specimens were analyzed for creatine, creatinine, and allantoin.

At intervals of one week, or oftener, blood was drawn from an ear vein of each monkey and examined by standard hematological techniques for erythrocytes, reticulocytes, hemoglobin, hematocrit, total white cells, and differential white cell counts.

RESULTS

After about 10 months on this diet the animals developed a progressive muscular weakness, which developed first in the hind quarters and progressed to the front limbs. In the extreme stage the animals experienced difficulty in getting to a sitting position. They tired easily and had difficulty in breathing. Even in the most extreme stage of the deficiency, however, they were able to eat.

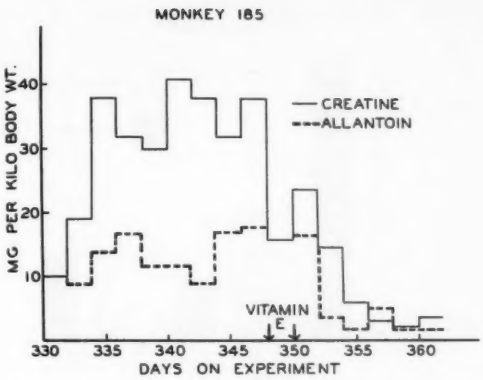

Fig. 1. Creatine and allantoin excretion by a deficient monkey.

Accompanying this muscular dystrophy were changes in the cytology of peripheral blood and in the amounts of certain nitrogenous constituents of the urine. Analyses of urine specimens of a typical animal are shown in Figure 1.

It will be seen that on the 330th day there was a sharp increase in creatine excretion and a moderate increase in the output of allantoin. This continued until the diet was supplemented with vitamin E (α-tocopherol) on the 348th and 350th days. This treatment with vitamin E was followed by an abrupt reduction in the output of creatine and allantoin. Both of these urinary constituents fell to normal levels.

Figure 2 shows in graphic form the *average* output of creatine, creatinine, and allantoin during the period of obvious muscular dystrophy. The diagonally striped bars represent the output of a normal animal, while the solid bars represent the output of a dystrophic animal. It will be seen that the deficient monkey showed a 30-fold increase in creatine excretion, and a 2.5-fold increase in allantoin excretion, but the output of creatinine was only one-half that of the normal animal.

Figure 3 shows the peripheral blood cell picture of a deficient animal. Normally, the total white blood cell count of a young monkey is between 10 and 20 thousand cells per microliter. The dystrophic monkeys consistently

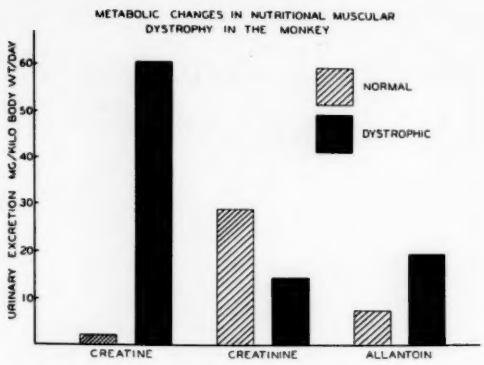

Fig. 2. Average daily excretion of creatine, creatinine, and allantoin, by a dystrophic monkey compared to a normal.

showed a moderate increase, frequently to values above 20 thousand per microliter. On the 348th and 350th days this animal was given a dose of vitamin E. This treatment was followed by a moderate decrease in the total white blood cells, a sharp decrease in granulocytes, and a moderate increase in lymphocytes.

The lower part of the figure shows the responses of the red cells of the animal. There was a mild anemia, characterized by an erythrocyte count of less than 3 million per microliter, a reduction in hemoglobin to approximately 8 grams per 100 ml of blood, and a reduction in hematocrit to 25 per cent.

Following the administration of two doses of 20 mg of α-tocopherol each, as shown by the arrows, there was a sharp increase in reticulocytes to 12 per cent, and a gradual return of erythrocytes, hemoglobin, and hematocrit to, or at least toward, normal levels.

The effect of vitamin E deficiency on the circulating blood cells is shown in Figure 4. In this bar graph the height of the bar represents the percentage of normal for each of the blood constituents. At the point in the experiment when the animals were definitely dystrophic the erythrocytes were reduced to approximately 50 per cent of normal, the hemoglobin to about 70 per cent of normal, and the hematocrit to 75 per cent of normal. However, the neutrophils were 250 per cent of normal, but the lymphocytes only 50 per cent of normal levels.

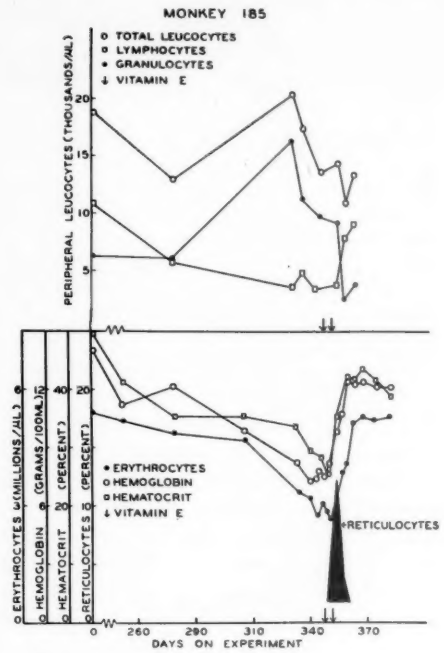

Fig. 3. Blood picture of a deficient monkey.

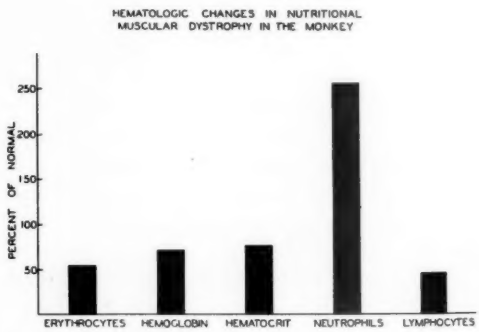

Fig. 4. Graph showing average blood counts of a deficient animal in comparison with normal values.

Muscle biopsies were taken of a dystrophic and a normal monkey. Histopathologic sections demonstrated clear evidence of muscle fiber degeneration in the deficient monkey as compared with the normal.

Moving pictures were shown which presented successively a normal monkey and then three stages in the development of dystrophy and its cure with α-tocopherol. "The normal young rhesus monkey is extremely active. When

the cage door is opened and the handler attempts to catch him, he jumps out of the cage and springs from the top of one cage to another, never seeming to tire. The second scene shows the earlier stages of dystrophy. When the cage door is opened the monkey attempts to hide in the corner. When removed from the cage he is able to crawl up the outside of the cage with difficulty. In the more extreme stage of the deficiency he has great difficulty in moving at all. When laid on his side on the floor he is able to struggle to a sitting position by pushing himself into a sitting position with his arms. He is able to crawl only with difficulty and his attempts to climb into his cage fail completely. However, 11 days later, following treatment with 40 mg of α-tocopherol in divided doses, his muscle strength has returned somewhat, and he is able to climb up the side of a cage, and into his cage with considerable agility."

SUMMARY

These experiments indicate that the young rhesus monkey is susceptible to vitamin E deficiency, showing a syndrome characterized by progressive muscular weakness, increase in urinary output of creatine and allantoin, but a reduction in urinary creatinine, progressive anemia, and leukocytosis. All of these physical, chemical, and cytological changes respond to the administration of α-tocopherol. We do not yet know what part the deficiency of vitamin B_6 may have in the development of the syndrome.

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DISCUSSION

Dr. R. E. Olson (Pittsburgh, Pa.): I was very fascinated by Dr. Day's paper because it represents the study of a multiple deficiency disease in which vitamin E is one of the absent nutrients. In our laboratory, we have undertaken a study of the biochemical pathogenesis of still another multiple deficiency disease in which vitamin E is one of the absent nutrients and cystine is the other. This deficiency disease is known as dietary hepatic necrosis and has been described by various investigators, including Daft of the National Institutes of Health, György of the University of Pennsylvania, Himsworth of the University of London, and Klaus Schwarz, currently of the National Institutes of Health.

We have found that animals fed diets deficient in these nutrients demonstrated marked changes in their hepatic carbohydrate metabolism prior to the onset of gross pathologic lesions. Although certain enzymes of the Krebs tricarboxylic acid cycle were found intact, the overall oxidation of pyruvate and acetate was found to be depressed. We found, further, that some of the coenzymes catalyzing reactions in the cycle were depressed, one of the most conspicuous being coenzyme A. We reasoned initially that this finding bore an important relationship to the pathogenesis of the disease, since dietary cystine probably served as the precursor for the thioethanolamine moiety of coenzyme A, and, in the absence of vitamin E, might become limiting for coenzyme A synthesis. We reasoned, further, that this limitation in coenzyme A synthesis could lead to biochemical ischemia and liver necrosis.

It has turned out, however, that the problem is not quite as simple as initially thought. We have studied the levels of coenzyme A in animals fed a diet low in organic sulfur (cystine and methionine) with and without supplemen-

tary vitamin E. In both cases, the coenzyme A content of liver is markedly reduced, in most instances there being no significant differences between the values for hepatic coenzyme A in animals receiving and not receiving vitamin E, even though only those on vitamin E-deficient diets go on to develop hepatic necrosis. If now, however, one attempts to define more functionally the metabolism of coenzyme A by a study of the rate of incorporation of S^{35}-labeled cystine into this coenzyme in these animals, one finds a marked difference in the animals on a necrogenic diet as compared to animals receiving the same diet with added vitamin E. The rate of incorporation of S^{35} from cystine into liver coenzyme A in the deficient group is approximately 33 per cent of that observed in the animals which are given vitamin E.

What, precisely, these data mean in terms of the relationship between vitamin E and sulfur-amino acid metabolism is not clear at this time. It may be related to the overall metabolic activity of the coenzyme which is reflected by an increased turnover rate of one of its components. This, in turn, may result indirectly from an action of vitamin E not presently defined. On the other hand, it may represent a defect in the synthesis of coenzyme A from organic sulfur precursors by the vitamin E-deficient rat which is matched by catabolic rates in such a way that the level is not affected.

The reason I felt that these data might be of interest to this group in connection with Dr. Day's paper is that we know the nutrient with which he has been concerned in addition to vitamin E, namely vitamin B_6, is also concerned in an important way with the metabolism of sulfur-amino acids. Specifically, pyridoxal phosphate functions as a coenzyme in the desulfuration of cysteine and the decarboxylation of cysteinesulfinic acid. It might be that an important function of vitamin E is to regulate sulfur-amino acid metabolism and that simultaneous deficiencies of cystine and/or vitamin B_6 result in additional widespread pathology.

Some Studies of Tocopherol in Infants and Children

By HARRY H. GORDON, M.D.* AND HAROLD M. NITOWSKY, M.D.†

IT IS our purpose to summarize data on the hemolysis of erythrocytes in hydrogen peroxide and on plasma tocopherol levels of infants and children. Studies were begun at the Colorado General Hospital during the last months of 1951 and have been continued at the Sinai and Johns Hopkins Hospitals in Baltimore for the past four years.

Our interest dates from the suggestion by the Owens, in 1949,[1] that defective absorption of fat by premature infants and the use of partially skimmed cow's milk mixtures[2–4] might lead to a deficiency of vitamin E. The reports by György and Rose[5,6] that hemolysis of erythrocytes in dialuric acid could be used as a measure of tocopherol deficiency in rats led to studies of dialuric acid hemolysis of erythrocytes of premature infants, with negative results.[7] Because low plasma tocopherol levels were being reported[1,8–10] for newborn full-term and premature infants, because the Owens' original suggestion still seemed reasonable, and because the variations in manifestations of tocopherol deficiency in different species had been stressed by Mason,[11] we did not believe the negative results with dialuric acid ruled out tocopherol deficiency. After György and his co-workers[12] reported that hemolysis in hydrogen peroxide could also be used as a test of tocopherol deficiency, we began our studies before publication of the method. With only slight modification, the test has proved technically satisfactory in our laboratories during the past four years.

Our first studies, made in a small group of premature infants fed partially skimmed or evaporated whole milk mixtures and in several adults, indicated that the red cells of the adults showed less than 10 per cent hemolysis, while for the majority of the premature infants, hemolysis was over 50 per cent.[13] The administration of tocopherol acetate was uniformly effective in reversing susceptibility to peroxide. Because it was not clear whether this evidence of tocopherol deficiency was a reflection of prematurity or of diet, studies were extended to other subjects: (1) newborn infants, full-term or premature, who had received no feedings or only glucose-water; (2) thriving young full-term infants who were studied in the well-baby clinic at an average age of seven weeks; (3) thriving young premature infants, less than three months of age, while still in the nurseries; (4) infants and children with steatorrhea, e.g. cystic fibrosis of the pancreas, biliary atresia, etc.

In addition, studies have been made in a group of mothers during the first post-partum day, and in healthy adult hospital personnel. Most of the data have been presented in detail elsewhere.[14,15]

HEMOLYSIS OF ERYTHROCYTES IN HYDROGEN PEROXIDE

Healthy Adults, Post-partum Mothers, and Unfed Newborn Infants

A summary of the results of hemolysis tests in 60 normal adults, 71 post-partum mothers, and 342 unfed newborn infants are presented in Table I. Virtually all adults showed hemolysis of less than 10 per cent, whereas only

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TABLE I

Hemolysis in Hydrogen Peroxide—Summary of Results for Adults and Unfed Newborn Infants

Subjects	No.	Hemolysis: Mean and stand. error	Hemolysis: Observations less than 10% hemolysis
		%[a]	%
Healthy hospital personnel[b]	60	2 ± 0.3	100
Post-partum mothers[b]			
Private white	23	2 ± 0.4	100
Service white	21	3 ± 0.6	100
Service Negro	27	7 ± 3.0	89
Newborn infants—unfed			
Full-term			
Private white	81	34 ± 3.2	26
Service white	133	43 ± 2.4	14
Service Negro	68	49 ± 4.0	16
Premature			
White[c]	42	35 ± 4.3	21
Negro	25	53 ± 6.1	8

[a] Per cent hemolysis is ratio of hemoglobin liberated from aliquots of red blood cells incubated in hydrogen peroxide and diluted with a buffer to that liberated by dilution in distilled water.

[b] No dietary surveys were made for any of these subjects.

[c] Lack of precise information prevented division of these infants according to economic status.

from 8 to 26 per cent of unfed newborn infants showed this resistance to incubation in hydrogen peroxide. No differences in mean hemolysis were found between full-term and premature infants in either the white or Negro groups. Significant differences were found, however, between the means for private white and service Negro full-term infants (difference ± S. E. diff. 15 per cent ± 5.16; $P < 0.01$) for private white and service white full-term infants (9 per cent ± 4.04; $0.01 < P < 0.05$) and for white and Negro premature infants (18 per cent ± 7.61; $P < 0.02$).

Thriving Full-term and Premature Infants Less Than Three Months of Age

In Table II is presented a summary of the results in thriving young full-term and premature infants. It is seen that at average age, seven weeks, both white and Negro full-term infants, who had been fed ordinary evaporated milk mixtures, showed significant decreases in

TABLE II

Hemolysis in Hydrogen Peroxide of Erythrocytes of Thriving Infants Less Than 3 Months of Age

Subjects	No.	Hemolysis: Mean and stand. error	Hemolysis: Observations Less than 10% hemolysis
		%	%
Full-term			
White			
Newborn—unfed	133	43 ± 2.4	14
7 weeks—cow's milk[a]	29	21 ± 4.1	55
7 weeks—breast milk[b]	7	4	100
Negro			
Newborn—unfed	68	49 ± 4.0	16
7 weeks—cow's milk[a]	25	31 ± 4.3	32
7 weeks—breast milk[b]	11	11	73
Premature			
White			
Newborn—unfed	42	35 ± 4.3	21
<30 days—cow's milk[c]	43	59 ± 4.9	9
30 days and over[c]	41	69 ± 4.5	5
Negro			
Newborn—unfed	25	53 ± 6.1	8
30 days—cow's milk[c]	31	62 ± 5.2	0
30 days and over[c]	22	53 ± 7.4	14

[a] The cow's milk mixtures consisted of ordinary dilutions of evaporated milk with added carbohydrate, estimated to contain 0.10 mg per 100 ml.[8]

[b] Human milk has been reported to contain 0.24 mg per 100 ml.[17]

[c] Although some of the infants were receiving unskimmed cow's milk mixtures at the time of testing, partially skimmed cow's milk mixtures, estimated to contain 0.04 mg per 100 ml,[8] had been fed during most of the time before the test.

mean hemolysis from 43 to 21 per cent and from 49 to 31 per cent, respectively (differences: 22 per cent ± 4.76; $P < 0.001$, 18 per cent ± 6.23; $P < 0.01$). The erythrocytes of breast-fed infants showed even less hemolysis; in all seven white and eight of eleven Negro infants hemolysis was less than 10 per cent, the level ordinarily found in adults. The spontaneous decrease in hemolysis on these diets which were supplemented only with vitamins A, D, and C is analogous to the spontaneous repair

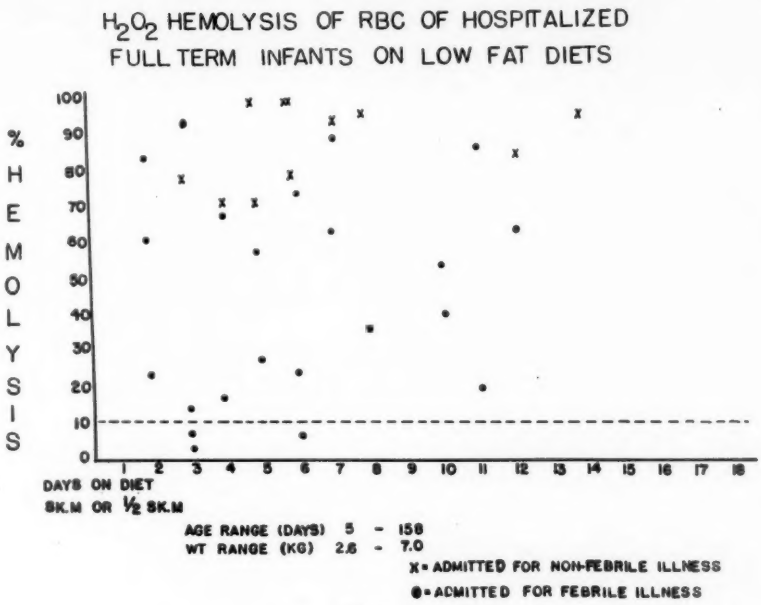

Figure 1

of neonatal hypoprothrombinemia without administration of vitamin K.

That young full-term infants fed cow's milk may have what Mason[16] has called a "precarious tocopherol status" is indicated by the data in Figure 1. Thirty-four observations were made on 17 young infants (age 5 to 15 days) admitted to the hospital because of febrile or non-febrile illnesses. The infants had all received unskimmed cow's milk before admission and completely or partially skimmed cow's milk mixtures after admission. Initial observations made from 2 to 14 days after beginning the low fat diets showed over 50 per cent hemolysis in 12 of 17 infants. One infant showed a rise in hemolysis from 1 per cent on the third day of the diet to 59 per cent on the seventh day; another showed virtually the same lack of hemolysis (2 and 12 per cent) on the eleventh as on the third day.

Erythrocytes of prematurely born infants fed partially skimmed cow's milk, which contains less tocopherol than either the evaporated cow's milk mixtures or breast milk[8,17] continued to show significant degrees of hemolysis. In view of their known defect in fat absorption, and the lower content of tocopherol in stored than in fresh human milk,[17] supplementation of their diets may be indicated even if one uses human milk in their feeding. We have been reluctant to recommend supplementation while we continue to search for some clinical, physiologic, or biochemical correlations of the deficiency.

Because the presence of positive hemolysis tests was related to low tocopherol intakes, infants and children with steatorrhea were studied. As has been previously reported,[18] these subjects also showed markedly positive tests. Thirty-eight observations have now been made in malnourished subjects with steatorrhea and 21 in subjects of comparable age, i.e., up to 15 years, with malnutrition due to other causes. In some of the children with steatorrhea a decreased fat intake had been prescribed, but this prescription was not being followed. Thirty observations, 79 per cent, showed over 50 per cent hemolysis, and four of the eight tests below 50 per cent were in patients receiving pancreatin. In the patients with malnutrition not associated with steatorrhea, only two observations showed hemolysis

TABLE III

Plasma Tocopherol Levels—Summary of Results

Subjects	Diet	No.	Plasma Tocopherol Mean and Stand-Error	
			mg/100 ml	
Adults				
Healthy hospital personnel	[a]	15	0.75 ± 0.040	
Post-partum mothers				
Private white	[a]	23	1.56 ± 0.080	
Service white	[a]	22	1.33 ± 0.052	
Service Negro	[a]	28	1.07 ± 0.060	
Infants				
Newborn				
Term	Unfed			
Private white	"	35	0.22 ± 0.003	
Service white	"	59	0.23 ± 0.015	
Service Negro	"	28	0.26 ± 0.023	
Total		122	0.23 ± 0.010	
Premature	"	14[b]		0.26 ± 0.042
Young Infants				
Full-term				
Ave. age 46 days	Cow's milk	55	0.33 ± 0.022	
Ave. age 46 days	Breast milk	14	0.72 ± 0.071	
Premature				
11–30 days	Partially skimmed cow's milk	17		0.20 ± 0.018
31 days and over		11		0.13 ± 0.024
Infants and children with steatorrhea[c]		31		0.18 ± 0.028

[a] No dietary surveys were made for any of these subjects.

[b] Nine were white and five were Negro.

[c] Subjects had proved cystic fibrosis of pancreas or biliary atresia.

over 50 per cent; these were in an eczematous infant whose mother had eliminated everything but a cereal-water mixture from his diet.

PLASMA TOCOPHEROL LEVELS

A summary of the results of 15 determinations in healthy hospital personnel, 73 in post-partum mothers, 136 in unfed newborn infants, 69 in thriving full-term infants, 28 in thriving premature infants, and 31 in infants and children with steatorrhea, are presented in Table III. The mean values for the healthy adults and for the post-partum mothers are in accord with previous observations[19–21] Significant differences were found between mean values for private white and service white mothers (differences ± S. E. diff. = 0.23 mg ± 0.096; $P < 0.01$) and for service white and service Negro women (0.26 mg ± 0.086; $P < 0.01$). No differences were found in the groups of unfed newborn infants, although such differences might have been expected from the hemolysis tests. No differences were found between full-term and premature infants, the levels for both groups being much lower than in mothers, in accord with previous findings[20,22,23] which suggest a relative impermeability of the placenta to tocopherol.

In the thriving full-term infants at average age of 46 days, the mean tocopherol levels had risen to 0.33 mg per 100 ml for infants fed whole cow's milk mixtures (difference = 0.10 ± 0.024; $P < 0.01$) and to 0.72 mg per 100 ml for infants fed at the breast. On the other hand, the premature infants fed partially skimmed cow's milk showed significant ($P < 0.01$) decreases in plasma tocopherol concentration with continuation of low tocopherol intakes. These results were to be expected from the findings on the hemolysis tests, from the reports of tocopherol concentrations in various milks, and from previous reports on tocopherol

values in both full-term and premature infants.

Thirty-one observations of plasma tocopherol were made in infants and children with steatorrhea. The mean level was only 0.18 mg per 100 ml, and all but two determinations were below 0.4 mg per 100 ml. These results confirm those of Filer[24] and Darby[25] and their co-workers.

CORRELATION OF HEMOLYSIS AND TOCOPHEROL CONCENTRATION

Since the hemolysis test has proved to be a simply performed index of deficiency of either tocopherol intake or absorption, an analysis has been made of observations in which simultaneous measurements of plasma tocopherol and erythrocyte hemolysis had been performed.

In 125 measurements in which plasma tocopherol was above 0.5 mg per 100 ml, hemolysis was below 10 per cent in 119, or 95 per cent. This relatively uniform lack of hemolysis of erythrocytes in hydrogen peroxide when the plasma tocopherol level is above 0.5 mg per 100 ml is in accord with *in vitro* studies,[26,27] as well as with the findings of MacKenzie in premature infants fed supplements of alpha-tocopherol acetate.[28] A summary of the correlations found when plasma tocopherol was below 0.5 mg per 100 ml is presented in Table IV.

It is seen that significant inverse correlations of from 0.5 to 0.7 were obtained for all but the unfed newborn infants. In these the plasma tocopherol concentrations were almost always low, but hemolysis occurred less frequently than in older infants and children with similar levels. This finding is a little ironic, since the first demonstration of positive hemolysis tests was in newborn infants;[12] its explanation is not clear. The data suggest that other factors than plasma tocopherol concentration may play a role in the hemolysis of erythrocytes in hydrogen peroxide, and these will be discussed in the next paper.[27]

TABLE IV

Correlation of Simultaneous Measurements of Hydrogen Peroxide Hemolysis and Plasma Tocopherol*

Subjects	Number of Observations	r	p
Patients with steatorrhea	63	−0.67	<0.001
Thriving full-term infants	69	−0.49	<0.001
Thriving premature infants	14	−0.71	<0.01
Unfed newborn infants	122	−0.03	>0.1

* Below 0.5 mg per 100 ml.
r = coefficient of correlation.

COMMENT

The studies of hemolysis and plasma tocopherol indicate, however, that newly born full-term and premature infants have a deficiency of tocopherol. In the former, it is remedied quickly by breast feeding and less consistently by feeding whole cow's milk mixtures, but in the latter it persists on customary feeding mixtures of partially skimmed cow's milk. Because of their defect in fat absorption and the limited tocopherol content of both whole cow's milk mixtures and stored human milk, supplementation of the diets of premature infants may be necessary to repair the deficiency. Using the average concentration of 0.24 mg per 100 ml of human milk reported by Harris, Quaife, and O'Grady[17] as a guide, one might estimate daily supplementation at 0.5 mg/kg. We are, however, reluctant to make such a recommendation while we search for correlates of the tocopherol deficiency both in these infants, and in the infants and children with steatorrhea who have prolonged deficiency.

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Some Studies of Tocopherol Deficiency in Infants and Children

III. RELATION OF BLOOD CATALASE ACTIVITY AND OTHER FACTORS TO HEMOLYSIS OF ERYTHROCYTES IN HYDROGEN PEROXIDE

By HAROLD M. NITOWSKY, M.D.* AND J. TYSON TILDON, B.S.†

IN A previous paper, an inverse relation was reported between plasma tocopherol and hemolysis of erythrocytes in hydrogen peroxide for infants and children with steatorrhea and for thriving young premature and full-term infants.[1] The absence of this expected relationship in unfed newborn infants led to the consideration that factors other than plasma tocopherol, e.g. differences in structure or composition of the neonatal erythrocyte, might affect its susceptibility to hydrogen peroxide. Since *in vitro* addition of purified catalase had been shown by Rose and György[2] to inhibit dialuric acid hemolysis of erythrocytes of vitamin E-deficient rats, and since some variation had been reported in the catalase activity of the erythyrocytes of newborn infants,[3] we studied the relationship between catalase activity and hydrogen peroxide hemolysis. In addition to these investigations, the *in vitro* effects of substances other than tocopherol on hemolysis have been determined.

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CATALASE

In preliminary studies, adult red cells, resistant to the hemolytic action of hydrogen peroxide, were incubated for 60 minutes with buffer solutions containing 10^{-3} to 10^{-5} molar 2,4-dichlorophenol, a specific inhibitor of catalase.[4] The cells were then washed and resuspended in saline, and hemolysis in hydrogen peroxide was determined in the usual way.[5,6] A typical experiment is presented in Table I. It is seen that inhibition of catalase by 2,4-dichlorophenol made the previously resistant cells susceptible to hemolysis in hydrogen peroxide.

TABLE I

Effect of Catalase Inhibitor on Hemolysis of Red Cells in Hydrogen Peroxide

Agent	Concentration	Hemolysis
	Molar	%
2,4-dichlorophenol	5×10^{-7}	2
"	5×10^{-6}	5
"	5×10^{-5}	45
"	5×10^{-4}	24
"	5×10^{-3}	94
Buffer control		2

Measurements of blood catalase activity in newborn infants were made by titrating with potassium permanganate the residual amount of hydrogen peroxide left after addition of a blood lysate. The lysate (1:1000) was added to 0.2 *N* H_2O_2 in 0.01 *M* phosphate buffer, pH 6.8 at 2–4° C, and the destruction of peroxide stopped by addition of 2 *N* H_2SO_4. The destroyed peroxide was determined at 0, 1, 3, and 5 minutes after addition of the

lysate and the pseudomonomolecular reaction rate constants were calculated. The initial catalase activity, K_0, was determined by extrapolation on a semilogarithmic plot of the reaction rate constants as a function of time. The exponential decline in the rate constants

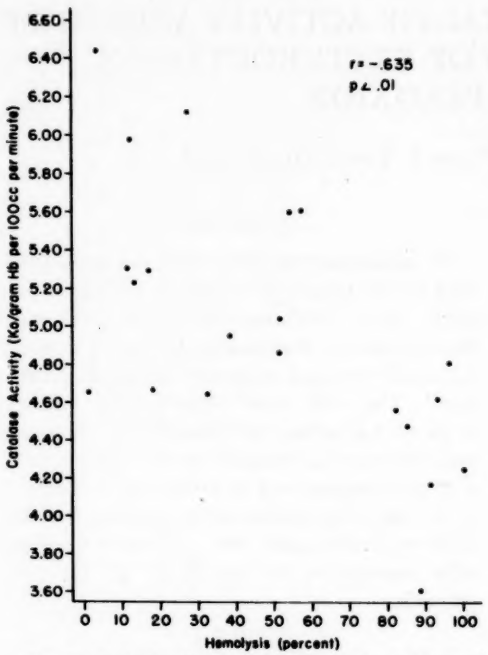

Fig. 1. Relation in unfed newborn infants between blood catalase activity and erythrocyte hemolysis in hydrogen peroxide.

resulted in a linearity which permitted easy extrapolation. The initial rate constant, K_0, was proportional to the hemoglobin concentration and was expressed in units per gram of hemoglobin per 100 ml per minute. The proportionality between K_0 and number of erythrocytes was not as satisfactory, perhaps because of errors in erythrocyte enumeration and differences in hemoglobin content of red cells.

Catalase activity in a group of 21 newborn infants was found to be inversely related to red cell hemolysis in H_2O_2 ($r = -0.635$, $P < 0.01$). No correlation was found in these infants between plasma tocopherol levels and catalase activity ($r = -0.072$), or between these levels and red cell hemolysis ($r = 0.032$). With a slight modification of method, observations have been made in an additional 44 unfed newborn infants. The inverse correlation (r) for the whole group of 65 infants is -0.474 ($P < 0.001$).

These results suggest that in newborn infants with low plasma tocopherol, high catalase activity may protect against hydrogen peroxide. Studies are being extended to older infants and children with low plasma tocopherols in whom the expected inverse relationship of the latter to hemolysis was found. Since this was already present in the young full-term infants at average age 7 weeks, and in the premature infants over 1 month of age, the relationship of catalase activity to fetal hemoglobin, which disappears during the early months of life, seems worthy of investigation.

EFFECT OF SUBSTANCES OTHER THAN ALPHA-TOCOPHEROL ON HEMOLYSIS

A variety of substituted hydroquinones and estrogens, in addition to several tocopherols, are capable of preventing *in vitro* hemolysis of the red cells of vitamin E-deficient rats by dialuric acid.[2] While fat-soluble antioxidants other than the tocopherols are inactive *in vivo*, the oral administration of methylene blue to tocopherol-deficient rats confers resistance to the *in vitro* hemolytic action of dialuric acid.[7]

We have determined the protective effects of a variety of substances added *in vitro* to newborn infants' red cells known to be hemolyzed by hydrogen peroxide. A tabulation of agents employed and the range of effective and ineffective concentrations are shown in Tables II and III. The method for testing these substances involved the addition of the desired amounts of the agent in 2 ml of phosphate buffer to 2-ml portions of a 5 per cent saline suspension of red cells. The tubes were incubated at 37° C for one hour and centrifuged; the supernatant was withdrawn and discarded and the cells were washed with 2 ml saline. A 5 per cent erythrocyte suspension in saline was then prepared, and the peroxide hemolysis test made in the usual manner. *d*-Alpha-tocopherol, α-tocopherol hydroqui-

TABLE II

Prevention by Various Substances of Hemolysis of Washed Erythrocytes by Hydrogen Peroxide

Effective agents	Range of effective concentrations
	μg/ml
Vitamins and derivatives:	
d-Alpha tocopherol	0.5– 10
Alpha-tocopherol hydroquinone	50 – 500
Thiamine hydrochloride	500 – 5,000
Sodium ascorbate	2500 –12,500
Miscellaneous agent	
Methylene blue	5 – 50
Quinones:	
2-Methyl-1,4-naphthoquinone	0.5– 50
1,4-Naphthoquinone	2.5– 25
1,2-Naphthoquinone	2.5– 25
p-Xyloquinone	0.5– 25
Tolu-*p*-quinone	50
2,5-Dichloroquinone	25
Quinone	2.5– 25
Hydroquinones:	
Hydroquinone	2.5– 25
3,4-Dihydroxyphenylalanine	25 – 250
1-Epinephrine bitartrate	25 – 250
Catechol	50

TABLE III

Failure of Various Substances to Prevent *in vitro* Hemolysis of Washed Erythrocytes by Hydrogen Peroxide

Ineffective agents	Range of ineffective concentrations
	μg/ml
Vitamin B Complex:	
Pyridoxine hydrochloride	5 – 500
Nicotinamide	5 –5,000
Vitamin B_{12}	0.05– 5
Riboflavin	5 – 500
Sodium folate	25 –2,500
Miscellaneous agents:	
Cortisone acetate	2.5 –2,500
Adenosine	5 – 500
Glutathione	0.5 – 500
Cysteine hydrochloride	0.5 – 500
DL-Methionine	0.5 – 500
2,3-Dimercapto-1-propanol	5 – 50
Resorcinol	0.5 – 500
Ephedrine sulfate	0.5 – 500
d-Amphetamine sulfate	0.5 – 500
Alpha-tocopherol esters	0.5 – 500
(Acetate, phosphate, carbosuccinate)	0.5 – 12.5

none, thiamine hydrochloride, and sodium ascorbate were found effective *in vitro* in preventing hemolysis. Methylene blue, and quinone, hydroquinone, and some of their substituted derivatives were also effective.

In preliminary studies, we have found no *in vivo* effects following oral administration of ascorbic acid or intramuscular administration of thiamine hydrochloride or the bisulfite salt of 2-methyl-1,4-naphthoquinone (water-soluble vitamin K); on the other hand, the positive *in vitro* effects of these metabolically useful substances suggest that they may condition the degree of correlation between plasma tocopherol of infants and hemolysis of their erythrocytes in hydrogen peroxide.

Of considerable interest were the *in vitro* tests of 2-methyl-1,4-naphthoquinone (menadione) and its salts. These substances were as effective as *d*-α-tocopherol on a molar basis. The addition of these agents to the red cell suspension caused a rapid formation of methemoglobin, as has been previously observed.[8] In spite of this oxidation reaction, a resistance of the red cells during subsequent exposure to hydrogen peroxide was noted. The *in vitro* protective action of water-soluble vitamin K derivatives is paradoxical in the light of a recent report that large doses of vitamin K cause hemolysis in vitamin E-deficient rats, and that kernicterus occurs in premature infants without isoimmunization after administration of large doses of vitamin K.[9]

Since the tocopherols function as reversible biological antioxidants, it has been proposed that a fundamental role of vitamin E is inhibition of fat oxidation.[10–13] Tappel[14] has found that α-tocopherol inhibits the hematin compound catalysis of unsaturated fatty acid oxidation and vitamin A and carotene co-oxidation. Other phenolic antioxidants also inhibit linoleate oxidation catalyzed by hemoglobin and cytochrome C.[15] It is of interest that several antioxidants which we have found to inhibit *in vitro* the hemolysis of susceptible red cells by H_2O_2 are reported to inhibit the activity of plant lipoxidase in catalysis of oxidation of unsaturated fatty acids.[16] Those substances which we found had no effect on hemolysis are similarly reported to have no effect on lipoxidase. A summary of these findings is presented in Table IV. The par-

TABLE IV

Comparison of Effects of Various Agents on Lipoxidase Activity and Erythrocyte Hemolysis

Agent	Inhibition of lipoxidase activity*	Reversal of erythrocyte hemolysis
Alpha tocopherol	+	+
Hydroquinone	+	+
Quinone	+	+
3,4-Dihidroxyphenylalanine	+	+
Epinephrine	+	+
Catechol	+	+
Ascorbic acid	+	+
Resorcinol	−	−
Sodium fluoride	−	−
Alpha tocopherol acetate	−	−

* Adapted from Sumner and Myrback.[16]

allel effects support the recently advanced hypothesis that vitamin E may play a role in maintaining the integrity of the erythrocyte by inhibition of oxidase action of hemoglobin on the unsaturated fatty acids of the cell membrane.[17] This role as a physiologic antioxidant need not preclude a more specific action of tocopherol through other enzyme systems, as has recently been demonstrated by Nason and Lehmann[18] in the enzymatic reduction of cytochrome C by reduced DPN (diphosphopyridine nucleotide).

CONCLUSION

Studies have been made of some factors which may be responsible for the variable susceptibility of the erythrocytes of newborn infants to the *in vitro* hemolytic action of a dilute hydrogen peroxide solution. Measurement of catalase activity in the red cells of these infants revealed a significant inverse correlation between this activity and erythrocyte hemolysis in H_2O_2.

A number of substances, some of physiologic importance, have been shown to be effective *in vitro* in reversing hemolysis of susceptible red cells. While no similar *in vivo* effects have as yet been demonstrated, it is postulated that some of these physiologic agents may influence the resistance of cells to hemolysis by H_2O_2 at low plasma tocopherol levels.

The correlation between the ability of various antioxidants to inhibit both hemolysis and catalysis of unsaturated fatty acid oxidation by lipoxidase or hematin compounds lends support to the hypothesis that vitamin E may play a role in maintaining the integrity of the erythrocyte by inhibition of an oxidase action on the unsaturated fatty acids of the cell membrane.

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DISCUSSION

DR. P. GYÖRGY (Philadelphia, Pa.): The hemolysis test in its detailed sequence is still shrouded in mystery, even after the excellent presentation of Dr. Gordon and Dr. Nitowsky. The hemolysis test *in vivo* is, at least in our hands, much more specific than has been claimed by some authors. This statement covers a large number of powerful antioxidants, given to rats in doses below the toxic level. The lack of real parallelism between the tocopherol level and the hemolysis test is not surprising. The catalase content of the red blood cells may be one interfering factor, as clearly demonstrated by Dr. Gordon and Dr. Nitowsky. The other possibility is that not all the tocopherol present in plasma is biologically active. It is conceivable that tocopherol bound on protein, in analogy to calcium bound on protein, is biologically inactive. Proof for this assumption is furnished by the observation that in the presence of serum more tocopherol is required to reverse a positive hemolysis test *in vitro*. In this connection, it is possible that fetuin, which is one special constituent of the plasma in the newborn, has a special affiinity for tocopherol.

With regard to the specificity of the hemolysis test, it should be mentioned that rats kept on the usual necrogenic yeast diet (with 18 per cent yeast) show a marked positive hemolysis test within a few weeks. By increasing the proportion of yeast in the diet from 18 to 40 per cent, the hemolysis test slowly becomes less intensive and may become negative. Yeast is free of vitamin E. In co-operation with Dr. Forbes and Dr. Zilliken, we have isolated a simple compound which has shown vitamin E-like activity on the hemolysis test *in vitro* and also *in vivo*. The relation of this substance to the vitamin E-like constituent postulated on the basis of studies on chicks by Scott and his associates at Cornell University, or to the Factor III of Schwartz, has to be further investigated.

The activity of alcoholic extracts from various yeasts on the hemolysis test seems to vary inversely with their necrogenic effect when fed as the sole source of protein to rats.

Alpha-Tocohydroquinone and Muscle Dystrophy

By PHILIP L. HARRIS, PH.D.* and KARL E. MASON, PH.D.†

AS PART of a continuing clinical study of myopathies, α-tocohydroquinone was tested for possible therapeutic effectiveness in patients with muscular dystrophy. The rationale was based on the similarity of muscle histopathologic changes present in dystrophic patients and in laboratory animals with nutritional muscular dystrophy induced by α-tocopherol deficiency. These skeletal muscle lesions are characteristic of vitamin E deficiency, having been observed in more than 20 species of laboratory and farm animals. In these animals, the muscular dystrophy can be prevented by administration of α-tocopherol or certain of its oxidation products. In the case of human dystrophy, α-tocopherol therapy is ineffective. Patients with progressive muscular dystrophy usually have normal levels of α-tocopherol in their blood and tissues. They have no difficulty in absorbing, transporting, and storing α-tocopherol; however, it has been postulated that such individuals might lack the ability to metabolize it *per se*, and that this metabolic defect or block could be bypassed by administering a partially metabolized form of α-tocopherol.

Of the degradation products of α-tocopherol which might be a normal-occurring metabolite and which could be prepared in sufficient quantities for a clinical test, α-tocohydroquinone was a likely candidate (Fig. 1). Alpha-tocohydroquinone has been altered chemically from α-tocopherol enough to destroy antisterility activity but it is very active in preventing muscular dystrophy in rabbits, according to Mackenzie and Mackenzie.[1] West and Mason[2] found it effective also in preventing muscular dystrophy in the hamster. They have developed a sensitive bioassay for antidystrophy agents based upon the relative numbers of degenerating and regenerating muscle fibers in muscle sections taken from dystrophic hamsters after treatment for 10 days with graded doses of test material. Furthermore, Milhorat and co-workers[3] reported that α-tocohydroquinone reduced creatinuria in patients with progressive muscular dystrophy.

Consequently, a clinical trial was arranged to test the working hypothesis that by administering α-tocohydroquinone the metabolic block in α-tocopherol metabolism might be bypassed and muscle functions returned toward normal.

METHODS AND MATERIALS

Subjects

Ten boys and two girls, ranging in age from 6 to 14 years and, with one exception, manifesting various phases of the Duchenne type of muscular dystrophy,[4] were used as subjects. Seven were assigned to α-tocohydroquinone therapy, and five to placebo administration. Each patient was given at intervals a bottle of capsules identified only by his name. Which patients received the drug and which the placebo was not disclosed until the end of the test. Examinations were

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‡ University of Rochester Medical Center and Edith Hartwell Clinic (Drs. R. P. Schwartz, F. Zuck, G. Forbes, P. Garvey, J. B. Shumway, and K. E. Mason).

These studies were aided by funds provided by the Muscular Dystrophy Associations of America, Inc.

made at six-month periods. Therapy continued for 12 to 18 months.

Alpha-Tocohydroquinone

The α-tocohydroquinone was prepared* by oxidation of *d*-α-tocopherol to α-tocoquinone followed by reduction to the hydroquinone form as shown in Figure 1. Gelatin capsules

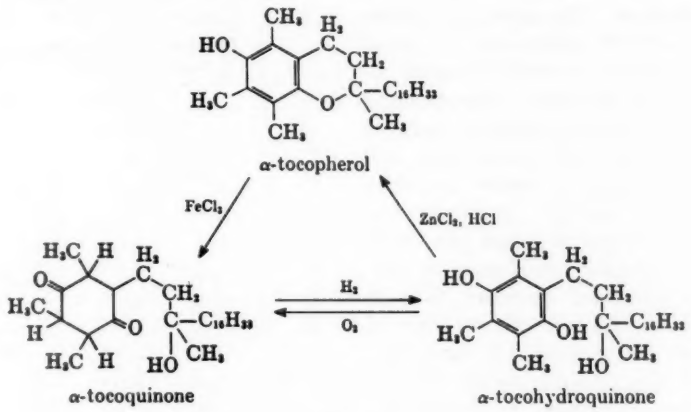

Figure 1

containing either 50 or 100 mg of the α-tocohydroquinone and placebos of identical appearance were made available for use.† Analysis of the capsules at intervals throughout the course of the test showed that the compound was completely stable.

Dosage

The dosage levels of α-tocohydroquinone varied from 200 to 600 mg daily and provided intakes for individual patients of approximately 7, 14, or 25 mg per kg body weight. The largest dose represented about one-half the dosage level, on a body weight basis, found to be minimum for curing experimental muscular dystrophy in vitamin E-deficient hamsters. In this connection, the data in Table I show the relative potency of α-tocopherol and various tocol derivatives as determined by hamster bioassay.[2] It is evident from the data in Table I that α-tocopheroxide, also a partially oxidized derivative of α-tocopherol, is about three times more effective than α-tocohydroquinone and deserves a clinical trial.

Efforts were made in two subjects to test the α-tocohydroquinone at levels of 50 mg/kg, which is commensurate with the effective dose in hamsters. However, a depression of prothrombin levels, which was not controlled by menadione (25 mg daily), necessitated return of the patients to the 25 mg/kg dose. The possibility of a prothrombin-lowering effect of α-tocohydroquinone in the patients had been anticipated, but not at levels as low as 50 mg/kg, as the result of the preliminary toxicity tests which had been carried out in rats. Massive doses (1000 mg/kg body wt.) had been found to cause internal hemorrhage, anemia, and death. Subsequent tests in which menadione was used at various levels as a protective anticoagulant showed that in the rat, vitamin K prevented the toxic manifestations resulting from large doses of α-tocohydroquinone and

TABLE I

Minimal Effective Dose for Repair of Dystrophy in the Hamster[2]

Substance	Amount
	mg
Alpha-tocopherol	2
Beta-tocopherol	4
Delta-tocopherol	>16
Alpha-tocoquinone	6
Alpha-tocohydroquinone	6
Alpha-tocopheroxide	2

* Distillation Products Industries, Rochester, N. Y.
† Eli Lilly & Co., Indianapolis, Ind.

that α-tocohydroquinone is, in fact, an antivitamin K. The antivitamin to vitamin ratio was determined to be about 1600 to 1 (700 to 1 on molar basis).

Criteria of Response

Five criteria were utilized in evaluating patient response to therapy: (1) creatine and creatinine excretion; (2) serum aldolase levels; (3) electrocardiograms; (4) motor-age muscle function tests; and (5) split-frame movies in Kodachrome, in which carefully planned sequences adapted to the motor limitations of the subject, and allocated to specified segments of 100-foot films, provided remarkably well synchronized recordings of motor activities prior to and after therapy. A device was inserted into the camera to mask the right half of the film during the filming of each subject as he performed exercises showing maximum muscular ability. After an interval of approximately six months, the same film was used (with the masking device reversed to protect the already exposed left half of the film) to photograph the same child performing the same exercise. The film was then developed and projected for evaluation. The left half of the picture on the projection screen showed the motor ability of the subject prior to, and the right half after, therapy. Slight changes in muscular ability could be observed by this technique.

RESULTS

In the dystrophic subjects, the degree of creatinuria increased quite markedly with increasing age and there was no appreciable change in the excretion of creatinine (Fig. 2). This is in contrast to values given for normal children of comparable ages[5] which show a slight decrease in creatinuria and a marked increase in creatinine excretion (Fig. 2). The ratio of creatine to creatinine excreted in the urine is a particularly good way of expressing this response since, as shown in Figure 3, children with muscular dystrophy are characterized by an increasing ratio with age, whereas the ratio for normal children actually decreases. Furthermore, only a single sample of urine, rather than a 24-hour specimen, is required for determination of creatine-creatinine ratio.

Serum aldolase levels were definitely above normal in all subjects (24 to 60 units compared to normal values of less than 10). This is in accord with the findings of Schapira and Dreyfus and their group.[6,7] However, the elevated values were not diminished by the

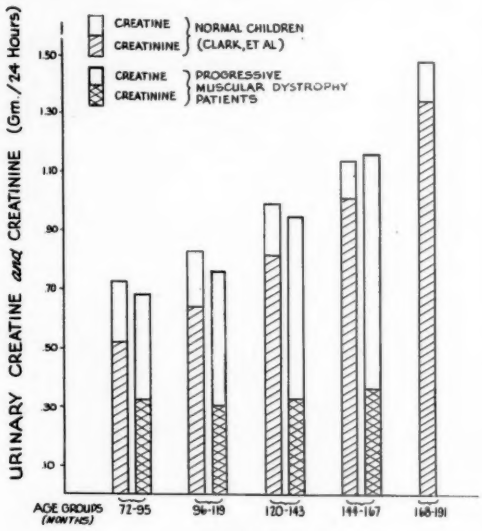

Figure 2

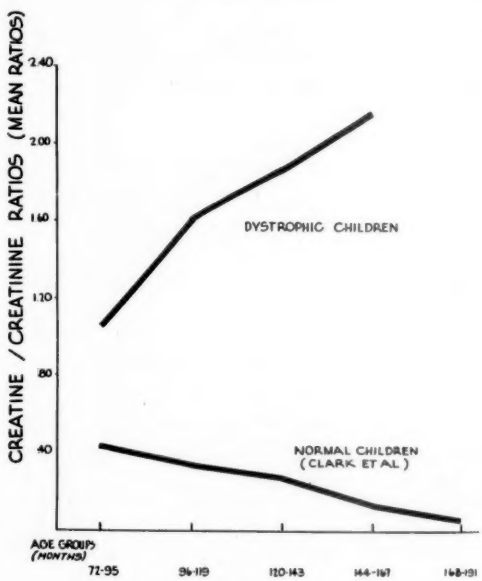

Figure 3

therapeutic measures employed in our subjects.

Motor-age muscle function tests, and routine testing of individual muscles and muscle groups, indicated no improvement with α-tocohydroquinone therapy over conditions existing prior to therapy. The electrocardiographic record showed no significant changes.

The recordings by split-frame cinephotography provided the most convincing and reliable evidence as to patient response, or lack of response, to therapy, and emphasized the merits of this technique in overcoming the fallacies of memory and in providing a permanent pictorial record for subsequent study and reference. In the case of patients maintained on therapeutic levels of 7 to 25 mg of α-tocohydroquinone daily, these records indicated a definite progress of the disease which, as far as can be determined, differed very little from that in the placebo group. Many of the sequences revealed unmistakable loss of muscle functions, sometimes combined with compensating attitudes. These losses were more evident in coarser movements of the extremities and trunk (walking, ascent and descent of stairs, bicycle riding, rolling over, crawling on hands and knees, going from prone to seated or to erect position, abduction at the shoulder joint, piling of blocks) than in activities requiring finer movements and co-ordination (picking up and placing of pegs in perforated board, writing, making of contacts for light bulb circuits).

SUMMARY

Alpha-tocohydroquinone at dosage levels of 7 to 25 mg/kg body weight for 12 to 18 months failed to influence the course of muscular dystrophy in children as measured by creatine excretion, serum aldolase activity, motor-age muscle function tests, and muscle function recorded by split-frame cinephotography.

Split-frame cinephotography has great merit in evaluating response, or lack of response, to drug therapy in progressive muscular dystrophy and in neuromuscular diseases in general. It also has great potential value in forming the basis of a permanent pictorial record of the course of progressive muscular dystrophy, and in analyzing the relative rate of loss of muscle function in different individuals suffering from the disease.

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DISCUSSION

Dr. C. Woodruff (Nashville, Tenn.): During the course of some 10 years that vitamin E levels in the serum have been done in the Division of Nutrition, we have determined vitamin E levels on about 175 patients that were seen in nutrition consultation. Among these were 28 patients who had serum tocopherol concentrations below 0.5 mg per 100 ml. A good many of these were cases of sprue that have been reported by Darby and Jones. There were four patients in this group who had practically no tocopherol in the blood. Two of these had celiac disease, one was a boy of 16 with Whipple's disease, and the last was a woman aged 42 years who had xanthomatous biliary cirrhosis of 10 years' duration. She had a severe defect in fat absorption, as manifested by steatorrhea, and a diminished ability

to absorb vitamin A. In conjunction with others at the Medical School, we have made some observations on this woman which are pertinent .

When she was first seen her serum tocopherol concentration was zero. No absorption followed the oral administration of 600 mg of α-tocopherol. Neither emulsification with Tween 80® nor the concurrent administration of bile salts resulted in the absorption of measurable amounts of tocopherol. She excreted creatine to the extent of 25 per cent of her total creatinine output. A pentose complex was present in her urine. She was treated with 100 mg of α-tocopherol daily emulsified with the aid of Tween 80. After three months of this therapy the creatinuria and pentosuria disappeared. After another three months of treatment the serum tocopherol level reached 0.34 mg per 100 ml. For the ensuing nine months she was given a placebo. The creatinuria and pentosuria reappeared and the serum tocopherol concentration fell to 0.06 mg. per 100 ml. Biopsy revealed a concentration of 0.236 mg. of tocopherol per 100 g of subcutaneous tissue, as determined in Dr. Karl Mason's laboratory. Hydrogen peroxide hemolysis of her red cells was increased at 34 per cent. She was given 250 mg of α-tocopherol intramuscularly in a water soluble form (*d*-α-tocopheryl polyethylene glycol 1000 succinate) supplied by Dr. Philip L. Harris. Since no increase was noted in the serum, she was given 2.1 g of α-tocopherol in the same form orally two days later. The serum concentration increased to 0.36 mg per 100 ml after six hours, and reached 0.64 mg per 100 ml after four days of such therapy. The creatinuria again disappeared, but the pentosuria continued to be present. The muscle biopsy made before this course of treatment showed extensive atrophy but the findings were not typical of avitaminosis E in the experimental animal and no "ceroid" pigment could be found. Because of her debilitated state no objective observations concerning her muscular strength could be made. These observations would suggest that at least biochemical evidence of vitamin E deficiency may be found in patients having severe defects in fat absorption of relatively long duration. (This case will be reported in detail in a future issue of this journal—Ed.)

DR. A. S. MINOT (Nashville, Tenn.): Ever since it has been shown by many investigators that muscular dystrophy can be produced in a wide variety of laboratory animals by withholding vitamin E from the diet, the idea has persisted in the minds of both clinicians and laboratory workers that there must be some basic fundamental common ground in clinical and vitamin E-deficient muscular dystrophy.

If you worked in this field as long ago as I did, you recall the heyday of clinical enthusiasm and hope which resulted when it was demonstrated that the various manifestations of nutritional dystrophy could be reversed by the administration of α-tocopherol. It then seemed reasonable to hope that the clinical condition might be equally successfully treated by such simple measures as the administration of various types of preparations of vitamin E. We need not rehearse the disappointments and failures which thus far have persisted throughout all attempts at influencing clinical dystrophy by simple therapy of this type.

In the papers we have heard here today the similarities between the two types of dystrophy have been adequately reviewed. The pictures we saw yesterday showing progressive muscular dysfunction in monkeys suffering from vitamin E deficiency were strikingly similar to the progressive deterioration seen in clinical cases of dystrophy. We have stressed also the histopathologic similarity of the changes in the muscles in the two types of dystrophy. The creatinuria which develops in the vitamin E-deficient animals and which has been discussed repeatedly as a useful criterion of the progress of the developing dystrophy is also characteristic of clinical dystrophy. Here, too, the progressively increasing weakness is accompanied by an increase in the urinary output of creatine.

There is another chemical analogy in the two conditions which has interested us here and which we reported in papers published some time ago. At the present time we do not know the significance of the observation.

We have, however, noted in some 20 or 30 clinical cases of muscular dystrophy that the urines consistently contain a reducing substance which is not fermentable and which is not one of the usual urinary constituents which may cause reductions with the usual sugar reagents. We finally succeeded in isolating this substance as an osazone which seems to meet the criteria of a pentosazone both as regards melting point and elementary analysis. Further studies with the help of Dr. Dziewiatkowski, who was here in the biochemistry department at that time, indicated that the reducing substance was probably being excreted as a pentose-phosphorus complex. The same material was also isolated from the urine of rabbits with vitamin E-deficient dystrophy. This is another superficial biochemical observation which is common to both types of dystrophy.

Then we come to the dissimilarities in the two conditions. Of course, the vitamin E-deficient animal has a very low serum tocopherol level. The tissues are depleted of vitamin E or tocopherol. This is in contrast to the fact that in clinical muscular dystrophy, as stated this morning, the levels of tocopherol are normal. In our series here we have found a range from about 0.7 to 1.2 mg of tocopherol per 100 ml of serum in untreated cases of clinical muscular dystrophy. Dr. Karl Mason and others have shown from studies of autopsy material from clinical cases that the tocopherol level in diseased muscles is as high or in some cases even higher than the levels found in normal muscles of persons of similar age. Then we have the tremendous differences in response to therapy. The vitamin E-deficient animal given adequate amounts of the vitamin promptly shows a decrease in the amount of creatine excreted and a gradual increase in muscle creatine as evidence of restoration of muscles. Less promptly, but gradually and eventually completely, the reducing substance we have described disappears from the urine of the treated animals as muscle function is restored. In marked contrast to this is the persistence of abnormal urinary findings and progressive deterioration of muscle function in cases of clinical dystrophy despite all our endeavours to furnish vitamin E in any form, or by any known route.

So if we continue to adhere to the hypothesis, and I think most of us do, that there is a common basic defect in the two conditions, there is a world of questions, of which you are much more aware that I am, which must be answered. In clinical dystrophy, is something blocking the utilization of, or in some way inactivating, the vitamin E which we find ready and waiting in dystrophic muscles? In our analyses of such muscles do we perhaps measure tocopherol which has been in some way altered so that it no longer functions as vitamin E? Of course, the wide variety of tocopherol compounds used in therapy represent attempts to circumvent any such possible inactivation.

We do not yet know the function of vitamin E in normal muscles. I think we suspect it is a necessary part of some enzyme system—perhaps a cofactor in one or several enzymes concerned with the metabolic release of energy for muscle contraction. In clinical dystrophy does vitamin E fail to get to, or to be built up into, these enzymes?

Progressive muscular dystrophy is often but not always congenital—I mean that while it is not always possible to obtain a familial history, in many instances you can. The disease is often listed as a congenital anomaly of metabolism. Is it beyond possibility that in the two types of dystrophy the defect is in the same enzyme system? May we not on the one hand have failure of the enzyme system because of lack of the necessary vitamin E, and, on the other hand, one due to a lack or defect in the apoenzyme of the same system? I am talking in a field that I know very little about, but it seems to me we should work very hard to find the answer to the fundamental question of what vitamin E does. I think we all have the feeling that if we ever find out the exact role of this vitamin in muscle metabolism we can then point our finger and say that this is the same step which is defective in clinical dystrophy. Whether it is defective for a reason that can be corrected, or whether the metabolic gap is one that we may hope to bridge by appropriate therapy, are questions we can't hope to answer until we know more about the gap we are trying to bridge.

Effects of Limited Tocopherol Intake in Man with Relationships to Erythrocyte Hemolysis and Lipid Oxidations

By M. K. HORWITT, PH.D.,* C. C. HARVEY,† G. D. DUNCAN,† AND W. C. WILSON‡

MAN'S need for vitamin E has not been established. The sponsors of the Elgin projects considered this problem of sufficient importance to assist in the organization of a long-term depletion study, which, like the three previous projects, is being conducted with the assistance of a committee§ of the Food and Nutrition Board of the National Research Council, for whose co-operation the authors wish to express their gratitude. As this is a report on work which is still in progress, parts of it are incomplete.

The basic questions to be answered are: (1) Is there a definite determinable requirement for vitamin E for the growth and maintenance of man that may or may not be related to autoxidations of tissue fat? (2) Is such a requirement for vitamin E a function of the amounts of oxidized unsaturated fat, other anti-vitamin E stress factors, or both, in the diet? (3) What, if any, are the manifestations of vitamin E inadequacies in man?

To support the recognized conception that α-tocopherol is a vitamin, one must agree that it is present in growing mammalian tissues, that it cannot be synthesized by the body, and that attempts to grow young mammals in its absence lead to distinct pathologic states. Nevertheless, despite intensive work by many laboratories, there remains a disturbing lack of positive information about man's need for vitamin E. Accordingly, Elgin Project No. 4 proceeds on the assumption that if a group of men are fed a diet low in vitamin E for a sufficient period of time, useful information might be obtained.

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Supported in part by a grant from The National Vitamin Foundation, Inc.

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GENERAL PLAN AND DIVISION OF SUBJECTS

As with all controlled studies planned to evaluate nutritional requirements of man, the cost in time and money is large, and patience is the investigator's most important asset. After a preliminary period of about six months, during which time subjects were selected, recipes were tested, and methods were evaluated and established, 38 male subjects were, in October 1953, divided into three groups as follows:

Group B. Nineteen subjects received the basal diet which contained less than 3 mg of tocopherol. Four of these subjects received 120 per cent and one, 80 per cent aliquots of all the ingredients of the basal diet in accordance with estimation of their needs, determined during the preliminary period.

Group BE. Nine subjects received the basal diet plus a supplement of 15 mg of *d*-α-tocopherol acetate per day. Three of these received 120 per cent and one, 80 per cent aliquots of the basal diet.

Group HD. Ten subjects received the hospital diet *ad libitum*.

The organization of the diet kitchen and the techniques used to control dietary intake are

TABLE I

Composition of Basal Diet

	Amount in various menus (g)								
Diet	I			II			III		
Menu	1	4	7	2	5	8	3	6	9
Breakfast:									
Cornflakes	30	30	30	30	30	30	30	30	30
Sugar	20	20	20	20	20	20	20	20	20
Bread	25	25	25	20	20	20	20	20	20
Milk	100	100	100	100	100	100	100	100	100
Butter	—	—	—	—	—	—	—	—	—
Rubarb	110	110	110	110	—	—	—	—	—
Jelly	40	40	40	20	20	20	20	20	20
Coffee	—	—	—	—	—	—	—	—	—
Dinner:									
Beef	100	100	100	75	75	75	100	100	100
Onions	25	25	25	25	25	50	50	25	50
Potatoes	144	144	—	144	—	144	—	144	—
Pickles, dill	25	—	—	—	—	—	—	—	—
Pudding, I	156	—	156	—	—	—	—	—	—
" II	—	—	—	—	150	—	—	—	—
Bread	25	25	25	20	20	20	20	20	20
Jelly	20	20	20	20	20	20	20	20	20
Butter	—	—	—	—	—	—	—	—	—
Cabbage	—	—	—	50	—	—	—	—	—
Jello	—	34	—	34	—	34	—	34	34
Cookies	—	—	—	50	—	—	—	—	—
Rice	—	—	45	—	45	—	45	—	45
Tomatoes	—	—	60	—	60	60	60	—	60
Apple streusel	—	—	—	—	—	—	204	—	—
Beets	—	50	—	—	—	—	—	—	—
Rhubarb	—	—	—	—	110	110	—	—	—
Turnips	—	—	—	—	—	—	—	105	—
Coffee	—	—	—	—	—	—	—	—	—
Tea	—	—	—	—	—	—	—	—	—

	Amount in various menus (g)								
Diet	I			II			III		
Menu	1	4	7	2	5	8	3	6	9
Supper:									
Rice	45	45	—	45	—	45	—	45	—
Tomatoes	60	60	—	60	—	—	—	60	—
Onions	25	25	25	25	25	—	—	25	—
Beets	50	—	50	—		—	—	—	—
Jello	34	—	34	—	34	—	34	—	—
Bread	25	25	25	20	20	20	20	20	20
Jelly	20	20	20	20	20	20	20	20	20
Corn	—	—	—	80	80	80	—	—	—
Pudding I	—	156	—	—	—	—	—	—	—
" II	—	—	—	150	—	150	—	—	—
Potatoes	—	—	144	—	144	—	144	—	144
Turnips	—	—	—	—	—	—	105	—	105
Cabbage slaw	—	—	—	—	—	—	105	105	105
Pickles, dill	—	25	25	—	—	—	—	—	—
Cabbage	—	—	—	—	50	50	—	—	—
Cookies	—	—	—	—	50	50	—	—	—
Apple streusel	—	—	—	—	—	—	—	204	204
Coffee	—	—	—	—	—	—	—	—	—
Tea	—	—	—	—	—	—	—	—	—
Additional sugar	60	60	60	40	40	40	50	50	50
" lard	22	22	22	22	22	22	11	11	11
Total lard added to menus	31	31	31	29	29	29	30	30	30

Calories per day	2214	2213	2143
Protein (g/day)	49	47	48
Fat (g/day)	60	56	56
Tocopherol (mg/day)	1.7	2.0	1.8

similar to those previously described.[1] The constituents of the three basal diets used are shown in Table I. Three different sets of recipes are available for each diet, so that, in effect, nine different daily menus are rotated in an effort to provide some variety to the regimen.

Consumption at 100 per cent levels provides approximately 2200 calories, 47 g protein, and 60 g fat. Vitamin supplements* are provided as follows: Vitamin A, 2500 IU; vitamin D, 500 IU; thiamine mononitrate, 0.8 mg; riboflavin, 1.11 mg; niacinamide, 12.8 mg; pyridoxine hydrochloride, 2.2 mg; *d*-panthenol, 3.8 mg; vitamin B_{12}, 11.0 μg; folic acid, 0.51 mg; and biotin, 0.058 mg. In addition, 30 mg of iron in the form of ferrous sulfate are given daily.†

The lard used in the diet is stripped of tocopherol by vacuum distillation in Rochester, N.Y.,‡ and shipped to Elgin in sealed cans containing about 400 g each. About 30 g stripped lard per day, or about half of the fat in the diet, is worked into puddings, apple streusel, cabbage slaw, soup, cookies, and potatoes.

EVALUATION OF TOCOPHEROL IN DIET

As the amounts of tocopherol in the total daily diets are at the lower limits of methods now available, a special effort was made to establish a satisfactory technique for its analysis. Recovery experiments were conducted at various extraction times and solvent concentrations to arrive at the choice of the following procedure, which will be published in detail elsewhere. Briefly, the method proceeds as follows:

To samples representing half portions of all the ingredients of a daily diet as fed, are added 5 g citric acid and sufficient ethanol to make the alcohol concentration of the mixture 20–25 per cent. The mixture (about 750–900 g) is then homogenized in a Waring Blendor and aliquots (about 50 g) taken for assay. These aliquots are mixed with sand in extraction thimbles and then extracted (Soxhlet) with three successive portions of anhydrous ethanol on a steam bath. The first two extractions are continued for two hours each, the third for four hours. The first two extracts, which include the water from the sample, are combined, the alcohol content adjusted to about 50 per cent, and extracted with 50 ml Skellysolve B: 40 ml of the Skellysolve fraction are evaporated under nitrogen and 80 per cent of the third extract is added to the residue. This 80 per cent portion of the combined extracts is saponified and the saponifiable mixture extracted according to the procedures described by Swick and Baumann.[2] The final steps in the assay utilize the procedures described by Quaife and Harris.[3] This method employs the Emmerie-Engel reaction after the elimination of interference by carotenoids and similar substances by catalytic (palladium) hydrogenation. Table II summarizes data obtained by applying this technique to the diets used in this study.

TABLE II

Tocopherol Analysis, 100% Diet

Menu	Number assayed	Tocopherol
		mg/day
1, 4, 7	21	1.7 ± 0.4
2, 5, 8	30	2.0 ± 0.4
3, 6, 9	27	1.8 ± 0.3
Average	78	1.9 ± 0.3

ANALYSIS OF URINE AND BLOOD

The quantitative tests performed include the evaluation of creatine, creatinine, nitrogen, pentose, and a rough estimation of the distribution of amino acids in urine. In addition to routine determinations of its tocopherol content,[4] plasma has been analyzed for total fatty acids, linoleic, linolenic, and arachidonic acids, cholesterol, vitamin A, and carotenoid substances. Hematologic examinations are made at approximately monthly intervals and include hemoglobin, hematocrit, and differential white cell count evaluations. Liver function tests performed include bromsulfalein retention time and plasma choline esterase determinations. In addition, tolerance

* Supplied through courtesy of Dr. E. Sevringhaus and Dr. M. Schiffrin of Hoffmann-La Roche, Inc.

† Supplied through courtesy of Dr. H. H. Howard of The Borden Co.

‡ Supplied by Distillation Products Industries, Inc., through courtesy of Dr. P. L. Harris.

curves for glucose, lactic acid, and pyruvic acid are obtained after the oral administration of glucose and the performance of a mild exercise test. The susceptibility of erythrocytes to hemolysis by hydrogen peroxide will be discussed in detail below.

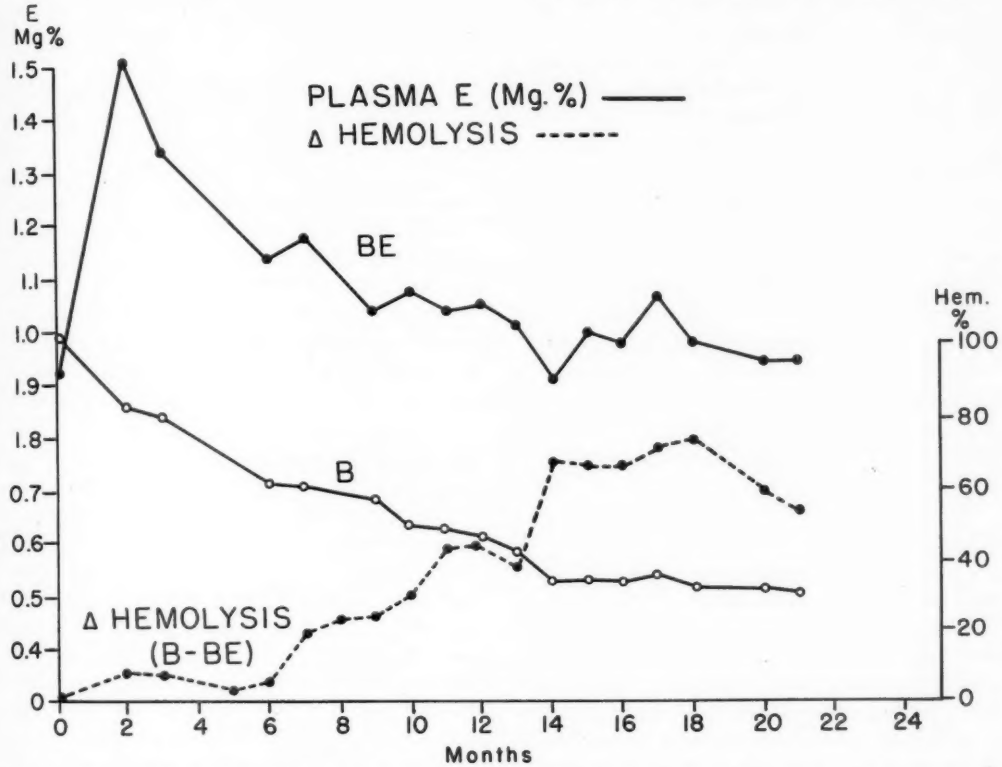

Fig. 1. Changes in plasma tocopherol of vitamin E-depleted group (B) and vitamin E-supplemented group (BE) related to the average increase in peroxide hemolysis of depleted subjects.

CLINICAL AND NEUROPHYSIOLOGIC PROCEDURES

The clinical and physiologic procedures used in this study include the Master test, recording of resting and exercise pulse rates, simultaneous recordings of electrocardiogram and fluorescine circulation times, the Rumpl-Leede fragility test, skin-fold caliper measurements, and basal metabolic rates. In addition, electromyographic base lines have been established in order to evaluate any changes which may take place in the future.

At this time (October 1955) there have been no significant variations in any of the tests applied to the human subjects in this study other than the changes in plasma tocopherol and the susceptibility of the erythrocytes to hemolysis by hydrogen peroxide. All tests continue to be performed as originally scheduled, in order to be prepared for changes which may develop at a later date. The remainder of this report will be devoted to a discussion of changes in plasma tocopherol and erythrocyte hemolysis.

PLASMA TOCOPHEROL

Figure 1 shows that there was a progressive decrease in the plasma tocopherol of the subjects on the basal diet for the first 14 months, after which time the rate of decrease was less pronounced. The average plasma tocopherol level had dropped to 0.64 mg per 100 ml after about one year. For the following 11 months, the average levels were 0.63, 0.61, 0.55, 0.53, 0.53, 0.54, 0.52, 0.52, and 0.51 mg per 100 ml,

respectively. The standard deviation was in all cases less than 0.08. Although most subjects tend to keep their rank order from month to month, the levels of some of them may vary about 25 per cent in successive months. This may be a consequence of the ebb and flow from the fat depots of the body. Several of the subjects were (in September 1955) beginning to show low levels not previously obtained in this study, e.g., one subject had a level of 0.33 mg per 100 ml at the last testing.

Referring to Figure 1, it is notable that the curve for the subjects receiving 15 mg of tocopherol in addition to the basal diet shows that there was a marked increase in the plasma tocopherol level during the early phase of the supplementation, but that this increase was not maintained. This is no artifact, as the same phenomenon has been noted in depleted individuals who were similarly supplemented. This phenomenon will be studied in greater detail at a later date when the subjects in group B are supplemented with various tocopherol derivatives.

ERYTHROCYTE HEMOLYSIS

The method as used at present is similar to that described by Rose and György[5] and by Gordon and de Metry,[6] except for the inclusion of a preliminary incubation period to standardize blood handling before delivery to the laboratory; the use of a higher temperature of incubation after the addition of peroxide; and the application of special precautions to keep the time relationships of each step identical, so that samples are treated similarly whether performed individually or in batches of eight or more at a time. The technique now used is as follows:

Whole blood which has been collected in about 20 volumes of citrate-saline solution is maintained for two hours at 25° C. The red cells are then precipitated by centrifugation and a 2.5 per cent suspension of these cells in phosphate-saline, pH 7.4, is incubated at 37° C for 15 minutes. The red cells are again separated by centrifugation and made up to a 5 per cent suspension of red cells in 0.9 per cent saline. Using suitable controls, 0.25 ml of the 5 per cent suspension are treated with 0.25 ml of 2.5 per cent peroxide in phosphate buffer. (The standardization of the peroxide addition is most important.) These tubes are incubated at 37° for three hours with mild agitation at 15-minute intervals to keep the red cells suspended. At the end of this incubation period the extent of hemolysis is determined in a spectrophotometer at 540 mμ by comparison with suitable controls.

Unfortunately, the method has the sensitivity of an immunologic technique rather than that of a chemical titration, and it has not always been possible to obtain the kind of quantitative reproducibility one would prefer. On any given day, there appears to be little difficulty arranging the various hemolytic tendencies in their correct rank order, but as a consequence of having to repeat the work on the same subjects month after month, technical variables have become apparent that would perhaps not be evident if different subjects were being tested routinely. It is our present opinion that one of the more important variables which has not yet been successfully controlled is in the standardization of the rate of peroxide decomposition when it is added to the blood sample. It appears that peroxide decomposition is affected by the age of the solution, the pH of the reaction, traces of heavy metals, traces of cleaning solutions on glassware, etc. Despite rather extraordinary precautions in the standardization of reagents and personnel, the test, which may give constant results for a three-month period, will unexpectedly give uniformly higher or lower data for a series of determinations. Fortunately, as subjects from the control experimental groups are always done simultaneously, we know when such a change takes place. Therefore, rather than give absolute values for the hemolysis data, a correction has been made in the data shown in Figure 1, which shows the difference in hemolysis obtained on a given day in blood from the subjects supplemented and unsupplemented with tocopherol. Before this refinement is misunderstood, it should be stated that for the usual clinical procedures such corrections may not be necessary, as the differences in the extent of hemolysis obtained between the control and experimental subjects are usually quite large.

An illustration of the dependence of the hemolysis test on the hydrogen peroxide used is given in Figure 2, which shows how the hemolysis of the erythrocytes of a depleted subject varied with the concentration of the 0.25 ml of peroxide used in the test. Note that at concentrations from 0.6 to 2.5 per cent the extent of hemolysis appears to be directly proportional to the peroxide concentration. The use of 5 per cent peroxide resulted in little more hemolysis, but at this concentration the color of the hemoglobin was altered. The 2.5 per cent peroxide concentration, which is close to the amount recommended by Dr. Gordon,[7] has been retained.

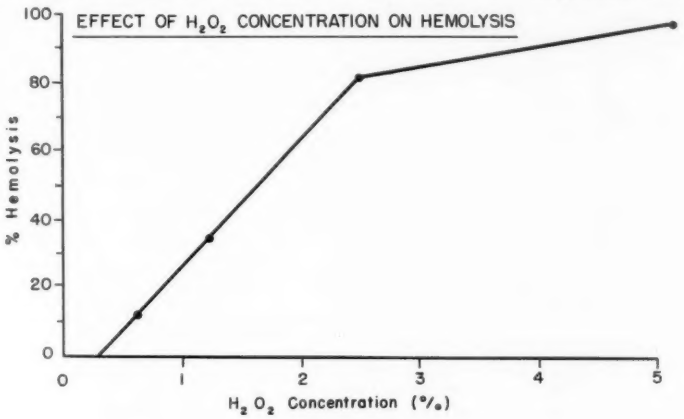

Figure 2

TABLE III

Hemolysis Obtained by Varying Rate of Addition of 0.25 ml of H_2O_2

Subject	All at once	In 2 parts 5 min. apart	In 6 parts at 1-min. intervals
BE5	1.0	5.5	31.
BE7	2.0	7.0	44.
B1	90.	90.	93.
B5	22.	56.	94.

Another illustration of the dependence of the hemolysis reaction on the hydrogen peroxide is given in Table III, the data for which were obtained by using the standard procedure, except that the method of peroxide addition was varied. This clearly demonstrates that adding a given amount of peroxide at different rates produces large variations in the per cent hemolysis obtained. The blood of four subjects, of whom two were being supplemented with tocopherol and two were from the depleted group, were used to show that rate of peroxide decomposition may be the most important variable in the method. In view of such data, it is important to standardize the type and area of surface in the test tubes used, and to reduce heavy metal contamination to a minimum.

Figure 3 shows the effect of changing the incubation temperature on a blood sample which gave relatively little hemolysis at 25°, even though the sample was taken from a depleted subject (B group). The curves marked BE and HD are for a tocopherol-supplemented

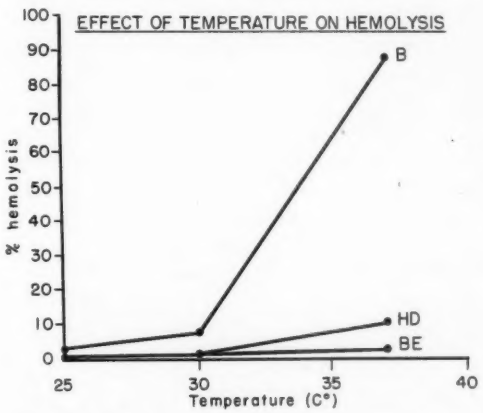

Fig. 3. Effect of varying incubation temperature after treatment of erythrocytes with peroxide. B, BE, and HD represent bloods from depleted, supplemented, and "hospital diet" subjects, respectively.

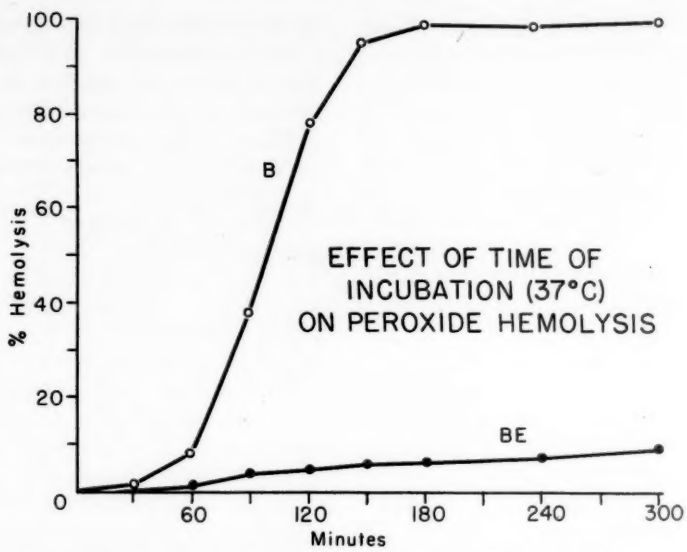

Fig. 4. Effects of varying time of incubation after treatment with peroxide of erythrocytes from depleted (B) and supplemented (BE) subjects.

subject and a subject on *ad libitum* consumption, respectively. Though the difference in hemolyses obtained at 25° and 37° C in this particular B group sample was unusually large, it does show how one may miss some important information if the lower temperature is used routinely. During the 16th month of the dietary restriction the blood of 13 subjects from the B group was tested, simultaneously, at both 25° and 37° C. The average obtained at 25° C was 23 per cent, as compared with 75 per cent at 37° C. Three of these subjects, who gave normal-appearing data of only 3.0, 2.0, and 5.2 per cent at 25°, gave hemolysis data of 24, 50, and 66 per cent, respectively, at 37° C. It is therefore strongly recommended that 37° C be used as the incubation temperature, after the addition of peroxide, instead of the 25° C level (room temperature) now routinely used in most other laboratories.

Figure 4 shows the effect of varying the time of incubation after the addition of 0.25 ml of 2.5 per cent peroxide to erythrocytes of a supplemented and depleted subject. Note that though at three hours one is closer to a plateau figure, most of the hemolysis is completed in 2.5 hours. These curves are reminiscent of the type obtained in studies on the oxidation of unsaturated fats. This relationship between erythrocyte hemolysis and the oxidation of fats will be discussed in detail below.

The effect of added tocopherol on the reaction was studied in the following manner: 2.5 per cent suspensions of erythrocytes from a depleted subject were prepared in buffer-saline solutions containing different amounts of tocopherol, as shown in Figure 5. These suspensions were incubated at 37° C for 15 minutes, centrifuged, and diluted to 5.0 per cent suspension in 0.9 per cent saline. The results of performing the peroxide hemolysis test on these erythrocytes show that the hemolysis obtained is inversely proportional to the amount of tocopherol added, and these amounts *in vitro* are quite consistent with the amounts normally present in blood. Similar experiments, which used propyl gallate instead of tocopherol, gave similar curves. This was also true of other fat-soluble antioxidants when added to erythrocytes from individuals on a low tocopherol diet. Compounds so tested included, diamyl-hydroquinone, butylated hydroxyanisole, 2,6-ditertiary-butyl-*p*-cresol, diphenyl-*p*-phenylenediamine, and nordihydroguaiaretic acid. The water-soluble antioxidants, ascorbic acid, glutathione, and cys-

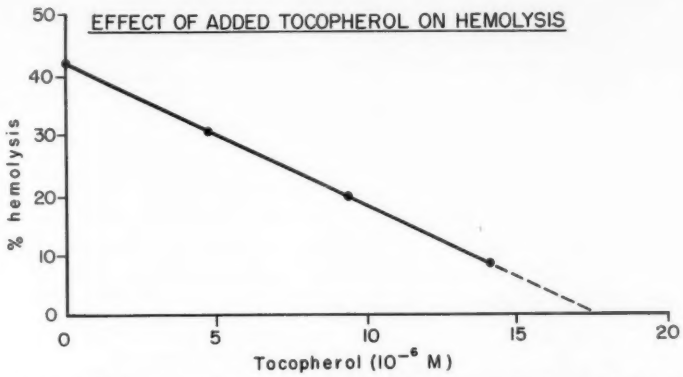

Fig. 5. Effects on hemolysis test of incubating blood samples from a depleted subject with varying amounts of alpha tocopherol.

teine, did not inhibit peroxide hemolysis at the low concentrations at which the fat-soluble antioxidants were effective. In interpreting the effects of the fat-soluble antioxidants on *in vitro* hemolysis, it should be remembered that all but the tocopherols may be considered foreign to the body and probably difficult to maintain in the living animal at effective concentrations.

SIGNIFICANCE OF HEMOLYSIS TEST

To date, no one has proved that the test in question is anything more than an *in vitro* assay of the antioxidant titer of the red blood cell, a test which could possibly have no direct relationship to the stability of the erythrocyte *in vivo*. Certain physiologic lysins may later prove to be inhibited by tocopherol, but at the present stage of our information this is pure conjecture. But more important, perhaps, than the possibility of tocopherol being an anti-lysin, is the fact that in the red blood cell one has an active physiologic system with a specific structure made up of oxidizable lipids that are easily available for a variety of multiplicate examinations. The stroma of the red blood cell is considered to be an organized complex framework. This framework, which is responsible for the characteristic doughnut-like shape of the erythrocyte, is composed of lipoprotein-like substances, specific for each species. In man, about 40 per cent of the total lipid in the red blood cell is cephalin, 21 per cent lecithin, 25 per cent cholesterol, 5 per cent cholesterol esters, and most of the remainder, about 10 per cent, a mixture of cerebrosides.[8]

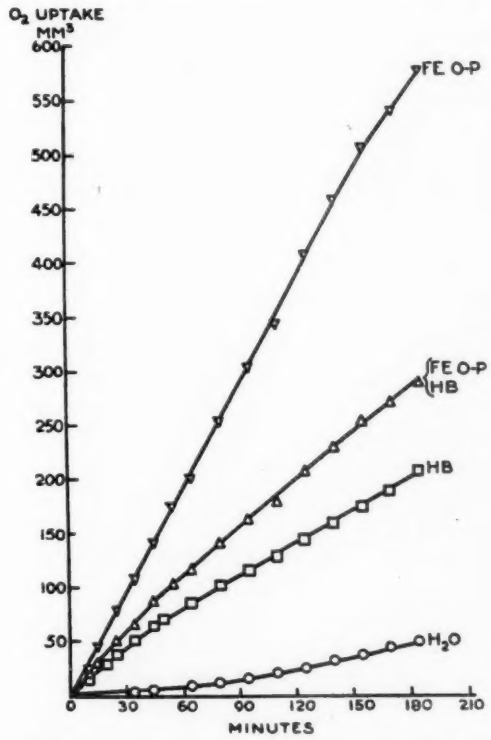

Fig. 6. Oxygen uptake of linoleic acid. ▽, with 0.0062 *M* ferrous-*o*-phenanthroline added; △, with 0.0062 *M* ferrous-*o*-phenanthroline and 1 per cent hemoglobin added; □, with 1 per cent Hb added; ○, H_2O control. (From Simon, Horwitt, and Gerard: *J. Biol. Chem.* 154: 421, 1944.)

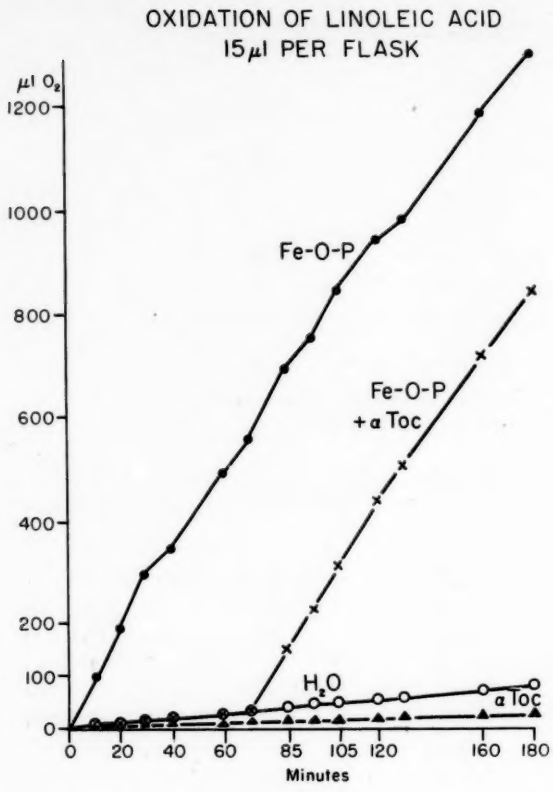

Figure 7

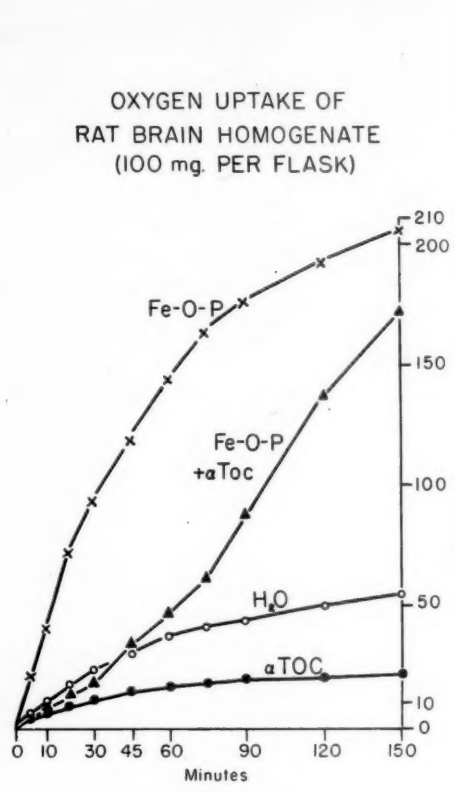

Figure 8

Fig. 7. Effects of alpha tocopherol (2×10^{-4} *M*) on "lipoxidase" effect of ferrous orthophenanthroline (4×10^{-5} *M*) on linoleic acid (15 μl per flask).

Fig. 8. Effect of tocopherol (6×10^{-4} *M*) on oxygen uptake of homogenized rat brain (H_2O) and on the acceleration of oxygen uptake of brain homogenate by ferrous orthophenanthroline (2×10^{-4} *M*).

The quantity of the protein component of the erythrocyte framework varies from 1.3 to 2.3 times the lipid.

From knowledge of the phospholipid nature of the stromata, one can make some preliminary interpretations of the manner in which oxidative processes affect the lipid structure of the erythrocyte by referring to some work on brain extracts done in this laboratory over 15 years ago.[9–11] At that time it was noted that a variety of iron-containing compounds, which included derivatives of α, α′-dipyridyl, orthophenanthroline, hemoglobin, and cytochrome c, all had marked catalytic, lipoxidase-like effects on the oxidation of linoleic acid and of the unsaturated lipids of the brain. In more recent years the role of tocopherol in inhibiting the oxidative effect of such compounds has been generally recognized.[12,13]

Figure 6 presents typical observations on oxygen uptake when the substrates in the Warburg flask are either brain homogenates, phospholipids from brain, or linoleic acid. Such oxidations of the unsaturated fatty acids are, as expected, inhibited by tocopherol, as shown in Figures 7 and 8* in which linoleic acid and brain brei, respectively, are used as substrates.

It is of interest to note, here, for later documentation, that when human erythrocytes were incubated for 1.5 hours with ferrous orthophenanthroline (1×10^{-4} *M*), the extent of peroxide hemolysis was greatly increased.

* Dr. B. Century of this laboratory performed these Warburg experiments.

THIOBARBITURIC ACID TEST

In recent years, a new test has been developed for estimating the oxidation of fats in food products by using 2-thiobarbituric acid (TBA),[14,15] which combines with compounds formed during fat oxidation to give red-colored products. As this test is very sensitive and apparently correlated with the formation and decomposition of fat peroxides, it has been used in an effort to obtain more information about the mechanisms of erythrocyte hemolysis. It is of interest that Kohn and Liversedge,[16] in 1944, showed that the reaction of oxidized tissues with TBA was inhibited by apomorphine, ergotomine, and epinephrine.

The stroma of red blood cells, partially purified by successive ultracentrifugations, when treated with hydrogen peroxide, gives, as expected, a much stronger color reaction than does stroma which is not so treated. When this test is applied to the intact erythrocytes of the experimental subjects and simultaneously correlated with the extent of peroxide hemolysis obtained, a distinct difference is noted between the reactions of the B and BE subjects. Table IV compares the intensity of the TBA test obtained on 9 subjects after their red blood cells were treated with hydrogen peroxide with the extent of hemolysis simultaneously obtained in blood aliquots similarly treated. Note that all of the depleted subjects had decidedly stronger TBA reactivities, as shown by the optical density of the color obtained.

When erythrocytes from depleted subjects were treated with tocopherol (2.4×10^{-5} *M*) there was a marked reduction in the intensity of the TBA test obtained. The details of these experiments will be presented elsewhere.

At this point in this discussion, it might appear that the hemolysis test is an uncomplicated assay of the amount of tocopherol present in the blood. Let us consider some evidence to the contrary. First, there is the lack of correlation between the level of tocopherol in the blood and the extent of hemolysis. Although all subjects in depleted groups have a higher hemolysis and a lower tocopherol concentration than the subjects in the supplemented groups, there is no positive correlation within each group. Secondly, when a depleted subject is supplemented with tocopherol it takes many months for the hemolysis test data to return to normal. In December 1954, one depleted subject was supplemented with 15 mg of *d*-α-tocopherol per day and another with 20 mg of *dl*-α-tocopherol per day. At this writing, after 10 months, although there has been a gradual improvement, these two subjects still show higher hemolysis figures than the subjects in the BE group who are receiving similar supplementation; this despite the fact that the plasma tocopherol levels of these two subjects were repaired within a few days after supplementation. For example, just before supplementation, the tocopherol levels and hemolysis figures for subject B14 were 0.48 mg per 100 ml and 61 per cent, respectively. At 1, 3, and 8 months after receiving 15 mg of *d*-α-tocopherol daily, these changed to 0.95 mg per 100 ml and 46 per cent, 0.70 mg per 100 ml and 52 per cent, and 0.85 mg per 100 ml and 40 per cent, respectively (Table V).

TABLE IV

Thiobarbituric Acid Test on Hydrogen Peroxide-Treated Human Erythrocytes before and after 2½ Hours Incubation at 37° C

Sample No.	Diet group	TBA* Optical density 5 min.	TBA* Optical density 150 min.	Hemolysis
				%
1	B	0.232	0.335	46
2	B	0.185	0.455	68
3	B	0.145	0.328	25
4	B	0.145	0.310	22
5	BE	0.300	0.220	0
6	BE	0.202	0.238	8
7	HD	0.165	0.202	0
8	HD	0.238	0.265	6
9	HD	0.197	0.240	0

* Optical density at 530 mμ, 5 and 150 minutes after the addition of 2.5 per cent hydrogen peroxide.

TABLE V

Effects of Supplementation of Depleted Subject with *d*-α-Tocopherol (15 mg/day)

Months	Plasma tocopherol	Hemolysis
	mg per 100 ml	%
0	0.48	61
1	0.95	46
3	0.70	52
8	0.85	40
9	0.90	12
10	0.95	10

In the 9th and 10th months, there were signs of a distinct improvement in the hemolysis test on this subject when, although the tocopherol had not changed significantly, there was a drop to 12 and 10 per cent hemolysis, respectively.

Another fact of some importance, although not directly related to the foregoing discussion, is the result of experiments being conducted simultaneously on the tissues of rats on diets containing varying amounts of tocopherol. Brains of rats, removed as rapidly as possible, were tested for their content of oxidized unsaturates by using the TBA test. Brain samples from rats deficient in tocopherol have shown a marked increase in oxidation products as compared to those obtained from vitamin E-supplemented animals. The physiologic significance of this observed difference in TBA reactivity in brain tissues remains to be determined.

SUMMARY

The organization of a project designed to evaluate man's requirement for vitamin E is described. This project is still in progress. The plasma levels of tocopherol have been studied in relationship to levels of vitamin E in the diet and compared with the extent of erythrocyte hemolysis by peroxide. A study has been made of variables which effect the hemolysis test. It is suggested that variables other than the concentration of α-tocopherol in the blood are involved in this phenomenon.

The hemolysis of the erythrocyte by hydrogen peroxide has been related to oxidation of the lipoid structure of the red blood cell by comparing data obtained from oxidation of linolenic acid, phospholipids, and brain lipids with oxidations of erythrocyte lipids.

Thiobarbituric acid (TBA) has been suggested as a suitable reagent for evaluating the oxidation of biological material in studies of vitamin E deficiency. With this reagent, data have been obtained which have shown an increased reactivity with biological material from mammals depleted of vitamin E. Correlations between TBA reactivity and the extent of peroxide hemolysis in human erythrocytes have been observed.

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For discussion see page 419.

DISCUSSION

DR. GRACE A. GOLDSMITH (New Orleans, La.): The experiment at Elgin seems a very logical one, namely, an attempt to determine whether vitamin E is needed in human nutrition. Most of the vitamins that have been found to be essential in experimental animals have been found to be needed by man. We know that vitamin E, or at least tocopherols, are present in the human body, and changes in tocopherol levels in the blood have been observed in association with variations in dietary intake and in certain pathologic conditions. Yesterday, someone remarked that vitamin B_6 nutrition had finally come of age. We might say that vitamin E, at least as far as human nutrition is concerned, is in the early embryonic stages of development, but that at least it seems to be showing some progress. There have been several flurries of clinical interest in vitamin E in the past. One was mentioned this morning by Dr. Harris and Dr. Minot. The similarity of muscular dystrophy in man to the dystrophy shown in experimental animals deficient in vitamin E led to attempts to treat human muscular dystrophy with this vitamin. Unfortunately, although everyone hoped for success, no beneficial results could be shown. Subsequently, when vitamin E was found to influence sterility in animals and deficiency was observed to lead to resorption of placenta and fetus, an application to human nutrition seemed possible. In controlled experiments, vitamin E was found to be without effect on human sterility and failed to prevent habitual abortion. Again, everyone was disappointed and vitamin E was dropped from clinical experimentation. Then, a few years ago, there was another flurry of interest in vitamin E when a report of its efficacy in the treatment of a large number of cardiac conditions was published. However, repeated attempts to substantiate these findings met with failure.

The current attempt to produce vitamin E deficiency in man seems to have considerable potential value, and there is a little encouragement, as well as some discouragement, from studies thus far. It is encouraging to know that in monkeys a long time must elapse before deficiency develops if vitamin E is the only nutrient missing from the diet. I believe Dr. Day said that it required two or three years, but that certain other dietary factors might influence the rapidity of onset. It would not seem desirable to produce a multiple vitamin deficiency in man, instead of vitamin E deficiency alone, but perhaps certain aspects of the diet at Elgin should be carefully examined. What is the vitamin B_6 content of the diet? What other factors in the diet may be sparing vitamin E, such as Dr. György's factor in yeast that was discussed earlier today? There may be several substances in this diet which are making it difficult to develop definite evidence of vitamin E deficiency. Furthermore, less than two years is not a long period of time when fat-soluble vitamins are concerned. Patience seems to be in order; certainly the experiment should continue. It will be desirable to search for various biochemical abnormalities that have been evident in several species of animals. It may be anticipated that some one of these abnormalities will eventually develop in man. Perhaps it will be necessary to use some antimetabolite, as has been done in studying vitamin B_6 deficiency in human subjects. It may be desirable to try some of the degradation or metabolic products of vitamin E such as those suggested by Dr. Harris.

The findings reported by Dr. Gordon and the case that Dr. Woodruff mentioned are of particular interest. They offer suggestive evidence that vitamin E deficiency may occur under certain physiologic or pathologic conditions. Perhaps, at some future date, we can say that vitamin E has come of age in human nutrition, but this certainly cannot be said yet.

Studies of Pantothenic Acid Metabolism

By ROBERT LUBIN, M.D.,* KATE A. DAUM PH.D,† and WILLIAM B. BEAN, M.D.‡
With the technical assistance of JAMES T. BRADBURY, M.S., RUTH GUNNING, M.S., JOSEPH I. ROUTH, PH.D. and I. CHU TUNG

IN PREVIOUS investigations of pantothenic acid metabolism in human subjects, Bean, Hodges, Daum, and Thornton[1–3] described metabolic abnormalities and clinical signs which occurred in normal young men given the pantothenic acid antagonist omega-methylpantothenic acid, and a diet devoid of this vitamin. The signs were those of an illness characterized by torpor, apathy, and depression; cardiovascular instability especially in the erect position; a neuromotor disorder with paresthesias, burning sensations and muscle weakness; abdominal pains and disturbance of alimentary function; and frequent infections. Biochemical alterations included an inconstant reduction in the percentage of *p*-aminobenzoic acid excreted in the urine in the acetylated form; irregularities in glucose tolerance and increased sensitivity to insulin; a failure of corticotropin (ACTH) to induce eosinopenia; an irregular reduction in 17-ketosteroid excretion; and the development of a histamine-refractory achlorhydria without any disturbance in gastric motility. The illness induced in the subjects caused us to abandon the planned recovery period, and to employ cortisone, a rich diet, and added vitamins. Because the abnormalities had not been anticipated, and because of the real but unmeasured hazard and the inherent difficulties in such studies in human subjects, the experimental design was not without limitations which are very obvious in retrospect. For these reasons it was desirable to repeat and extend the observation in additional subjects.

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EXPERIMENTAL DESIGN

We planned the present study with more elaborate control periods (Fig. 1). Period I was designed to establish baselines and the routine tests while employing a general hospital diet; Period II was to test the adequacy of the formula with mineral and vitamin supplements and the effects of intubation, since the formula was intolerable by mouth; Period III was designed to see whether the deficient diet, without the antagonist, might induce changes. Each of these periods in plan and in fact was two weeks long. Period IV with the deficient diet plus 500 mg of ω-methylpantothenic acid, was planned for four weeks. At the end of this time we extended it for one week because the clinical state of the subjects suggested that they had not reached a danger period. Their symptoms of illness were not as striking as those in the earlier study after four weeks of deficiency. Because 10 weeks is about the limit of endurance in taking tube feeding, the planned recovery Period V, with the formula plus pantothenic acid but without the antagonist, was reduced from two weeks to a single week. During this week the general condition of one subject improved, whereas the other had a progressing emotional disorder. Only when the chemical data were assembled did we find that some of the changes had not returned to normal till Period VI, when a normal diet, together with vitamin supplements, was re-instituted.

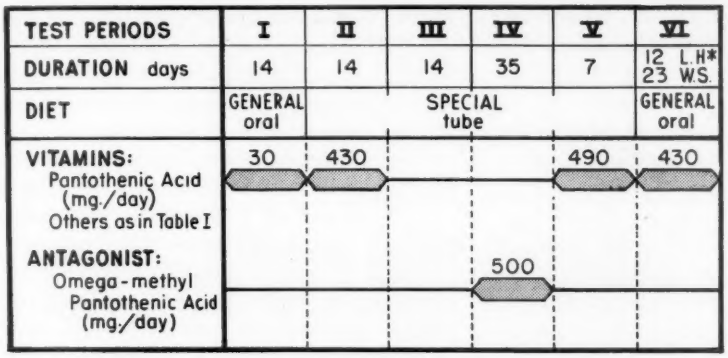

Fig. 1. The experimental design: test periods, diets, vitamins, and antagonists

PROCEDURES

The formula and vitamin and mineral supplements are indicated in Table I. The pattern is similar to that reported previously.[1–3] The calorie intake in Periods I and VI was the same as during the time the formula was used. W. S. was the only normal subject on whom we collected complete data. In another young man the experiment was stopped after two weeks of Period IV because of a breach of diet control. The other subject, a 31-year-old white woman, had obesity and the adrenogenital syndrome. We wanted to see whether the antagonist and deficient diet might reduce her adrenal cortical overactivity. Also, we used a 1000-calorie reducing diet throughout the period of the study. She received only a third of the quantity of formula but the same supplement of vitamins and minerals.

In our present studies we concentrated on fluid and electrolyte problems while repeating many of the studies we had done previously.

The original plan was somewhat different for the patient L. H. Her control period on a general diet lasted from January 12, 1955, to March 7, 1955, a period of eight weeks. In all periods she had only 1000 calories a day. On March 7 she started the pantothenic acid-deficient tube formula and 500 mg of ω-methylpantothenic acid. She then had a five-week period exactly like that of Period IV in the case of W. S. Period V likewise was identical

TABLE I

Formula and Vitamin and Mineral Supplements

Composition of formula		Vitamin supplement		Composition of salt mixture	
			mg		*mg*
Granulated sugar	290 g	Thiamine hydrochloride	1.2	Calcium biphosphate	67.9
Cornstarch	75 g	Riboflavin	1.8	Calcium lactate	163.5
Water	750 ml	Pyridoxine	2.0	Ferric citrate	14.9
Vitamin-free casein*	125 g	Ascorbic acid	50.0	Magnesium sulfate	68.5
Corn oil	90 g	Niacin	12.0	Dibasic potassium phosphate	119.9
Cystine	750 mg			Sodium biphosphate	43.6
Vitamin A	5330 U.S.P. units			Sodium chloride	21.8
Vitamin D	1070 U.S.P. units				
$NaHCO_2$	10 g				
Calories	3000				

* Contains 20 μg vitamin B_{12} by assay.

We were enabled to do the study with prisoners as volunteers through the thoughtful co-operation of Mr. Roy Purcell, warden of the Iowa State Reformatory at Anamosa, and with the authorization of the State Board of Control, Mr. Henry W. Burma, Chairman.

with that of subject W. S., as was Period VI, with the exception that in the case of L. H. it was of only 12 days' duration, for W. S. it was of 23 days' duration.

RESULTS

Clinical

H. T. had no symptoms until the beginning of the second week of Period III. Then for the first time he noted increasing fatigue, slight weakness, and transient unsteadiness upon standing upright. These symptoms, although non-progressive, were unrelenting. No personality change was noted: he remained pleasant and co-operative. Four days after the start of Period IV, H. T. first noted flexor spasms of the right forearm and hand. Three days later, paresthesias of the upper and lower extremities began. Repeated neurologic examination failed to reveal any abnormalities until eight days after the start of Period IV, when a slight but definite decrease occurred in the tendon reflexes on the right side. This progressed for three days and then remained stationary. No other symptoms or abnormal signs appeared.

Subject W. S. was asymptomatic until the beginning of the second week of Period III. Then he noticed the onset of increasing fatigability, a generally tired-out feeling, mild weakness, and unsteadiness of gait on arising in the morning. Three days after the beginning of Period IV, he had cramps of the right anterior thigh muscles and severe pain in the right Achilles tendon. At the end of the first week of Period IV paresthesias of the upper and lower extremities began. By the next day he was extremely torpid; and for the first time the tendon reflexes on both sides had decreased. Otherwise the neurological examination and physical examination were normal. Two weeks after the start of Period IV W. S. was troubled by severe muscular spasms of his hands, forearms, and legs. Although the paresthesias persisted there was no muscle tenderness. He felt that his overall strength was improving. A week later he had less muscular cramping. The paresthesias persisted, he was much more lethargic and somnolent, and spent most of the time in bed. It was only with difficulty that he could be aroused in the morning. After four weeks of Period IV, for the first time a positive Trousseau sign was demonstrated and the tendon reflexes became still more inactive. Two days before the end of Period IV he had repeated bouts of nausea, increasing somnolence, and moderately severe muscle cramping and paresthesias. One day after the start of Period V nausea became troublesome. It was only with difficulty that he could retain the tube feeding. Only 24 hours after Period VI was begun there was complete cessation of the paresthesias in subject W. S. and he became less somnolent. A week later the muscular cramping was gone and the Trousseau sign was negative. He felt wonderfully well. All lethargy, somnolence, and weakness were gone, although diminution of tendon reflexes remained.

L. H. remained asymptomatic until 11 days after the start of Period IV, when she had muscular pains in the legs, calf muscle tenderness, and slight unsteadiness of gait. No definite reflex changes were demonstrable at that time. Seventeen days after beginning Period IV she had intermittent spontaneous carpopedal spasm in addition to severe "menstrual cramps" without bleeding. Thigh and calf muscles were extremely tender. Paresthesias had developed in the lower extremities. The remainder of the neurologic examination was negative. After 23 days of Period IV she noted some lessening of the muscular cramps but the paresthesias persisted along with a definite increase in lethargy and somnolence. During the last week of Period IV she was nauseated and refused her tube feeding on four occasions. After four days of Period V her clinical status was unchanged except that some disorientation and hallucination occurred. She kept saying, "People are taking pictures of me." During the last few days of Period V she became extremely depressed, cried frequently, and often repeated, "I want to kill myself." After five days of Period VI she was completely asymptomatic and she felt better than she had for many months. No abnormalities of the tendon reflexes could be demonstrated.

No significant lability of pulse rate or change in blood pressure or pulse pressure occurred in any subject. Electrocardiograms failed to reveal any abnormalities in subject H. T. until the beginning of Period IV when the U-waves in the precordial leads became prominent. reported previously.[3] During Period IV none of the subjects had upper respiratory infections or other manifestations of decreased bacterial resistance. W. S. and L. H. had a progressive increase in the sedimentation rate after the beginning of Period IV (Fig. 3).

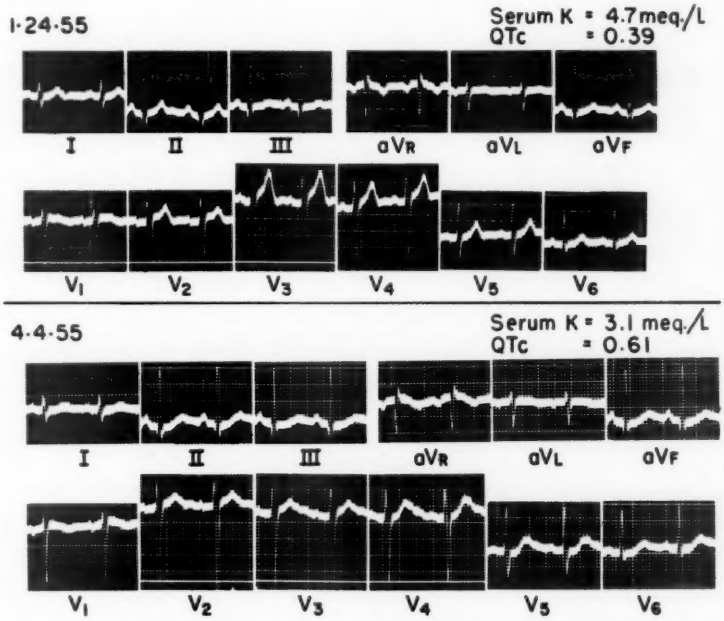

Fig. 2. The electrocardiograms before and during hypokalemia (Patient W. S.).

In W. S., repeated electrocardiograms were normal until the beginning of the second week of Period IV at which time T-wave changes and U-waves in the precordial leads were those seen in hypokalemia (Fig. 2). In L. H. control electrocardiograms revealed a prolonged QT_C interval and abnormal T-waves. As the experiment progressed she also developed abnormalities.

Ballistocardiograms of H. T. were normal until the first week of Period IV when definite abnormalities appeared. In patient L. H. control ballistocardiograms of H. T. were normal until the first week of Period IV, when definite abnormalities appeared. In patient L. H. control ballistocardiograms revealed bizarre complexes, but in W. S. no abnormalities developed.

Chest films failed to reveal any alterations. Gastrointestinal changes were similar to those

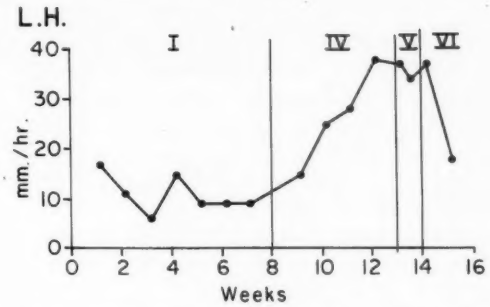

Fig. 3. The increase in erythrocyte sedimentation rate (Westergren method).

It promptly returned to normal early in Period VI. No alteration in the sedimentation rate of subject H. T. occurred during the control period, but it increased after one week of Period IV. During the experiment there was no significant change in the weight of subjects

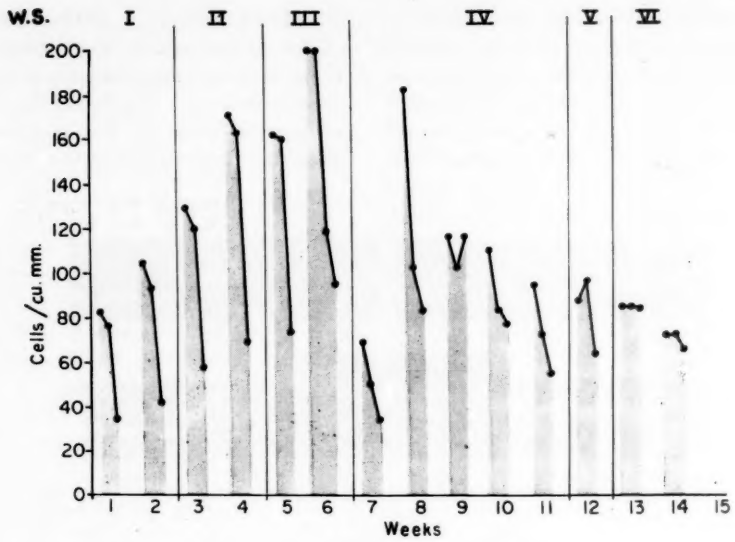

Fig. 4. The eosinopenic response to ACTH.

W. S. and H. T. L. H. lost weight on the 1000-calorie diet at the same rate in all periods. No significant alterations were noted in the hemoglobin, red blood count, white blood count, or urinalysis. Weekly eosinophil counts failed to reveal any significant variation.

METABOLIC RESPONSES

Eosinopenic Response to ACTH

In one subject, H. T., no significant alteration was noted. In both L. H. and W. S. there was a definite decrease in the eosinopenic response to ACTH beginning in Period IV and continuing through Periods V and VI (Fig. 4).

Kepler-Power-Robinson Test

Subject H. T. had one abnormal test during Period II, but all other tests were normal. W. S. had an abnormal test during Period I and II, but subsequently it became and remained normal. A striking aberration occurred in L. H. Numerous tests during the control period were all well within normal limits. During Period IV she developed an extremely abnormal response, as manifested by an inability to excrete the water load. The test remained abnormal until after the start of Period VI (Fig. 5).

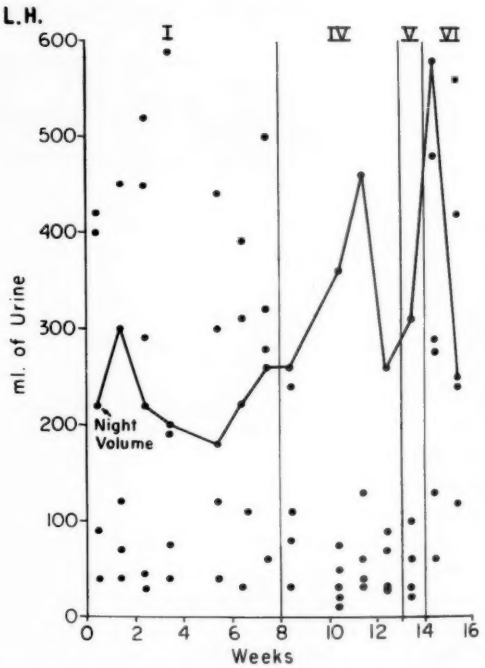

Fig. 5. Water excretion test (Kepler-Power-Robinson test)

Para-aminobenzoic Acid Acetylation

Acetylation of a standard dose of PABA did

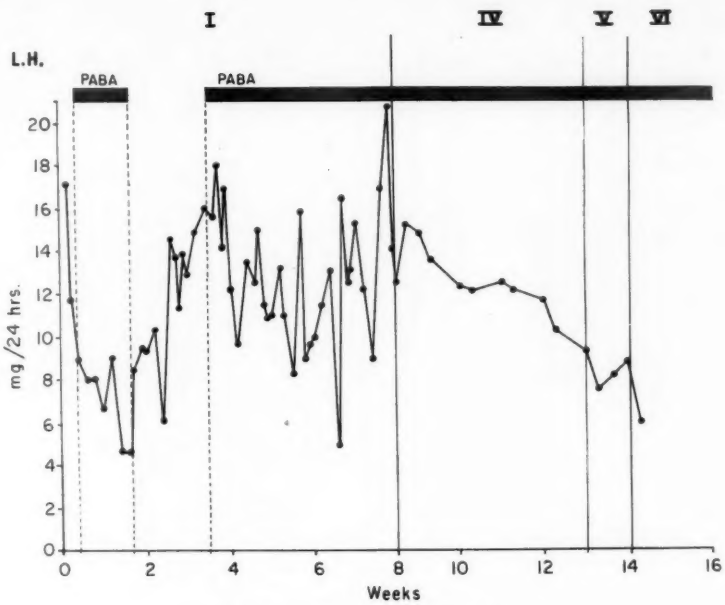

Fig. 6. Acetylation of PABA.

Ratio: $\frac{\text{17-ketosteroids}}{\text{creatinine}}$ in urine

not change significantly in any subject throughout the study.

17-Ketosteroid:Creatinine Ratio

In subject H. T. no significant change occurred. In W. S. a definite decrease in the excretion of 17-ketosteroids occurred toward the end of Period IV. It persisted through Period V and the early part of Period VI. Before the study L. H. had a greatly increased 17-ketosteroid excretion. At the beginning of the control period *p*-aminobenzoic acid (PABA) was given to test acetylation. As soon as PABA was given there was a prompt fall in the urinary excretion of 17-ketosteroids (Fig. 6). It again became markedly elevated when PABA administration was stopped. While again excreting large amounts of 17-ketosteroids in the urine, PABA was again started. This was followed by a slight but definite decrease in the amount of excreted 17-ketosteroids, which nevertheless was still above normal. A definite decrease from this level was noted in Periods IV and V, as well as Period VI, in this subject.

Glucose Tolerance Test

In W. S., marked alterations occurred. Although he had an abnormality of his glucose tolerance test throughout the entire study, during the latter part of Period III and throughout Period IV, a new abnormality occurred (Fig. 7). This was characterized by a persistent elevation in the two-hour blood sugar determination. This alteration was lost promptly during Period V and did not recur. Likewise, in L. H. a similar alteration occurred. During Periods IV, V, and the early part of Period VI, an elevation of the two-hour blood sugar occurred. The curve returned to the control form after the first week of Period VI.

Insulin Tolerance Test

In subject H. T. an increase in sensitivity to insulin was noted during Periods III and IV, with the 20-minute blood sugar much lower than in the control tests. In subject W. S., a marked increase in sensitivity to insulin began in Period III and persisted through Periods IV and V (Fig. 8). Subject L. H. likewise had a great increase in insulin sensitivity.

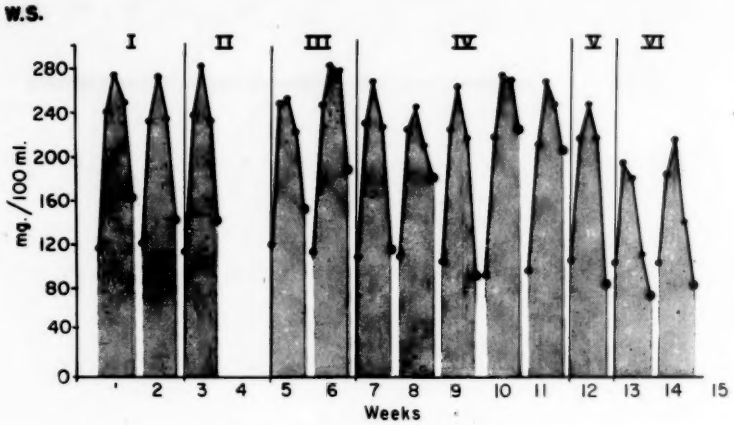

Fig. 7. Glucose tolerance test.

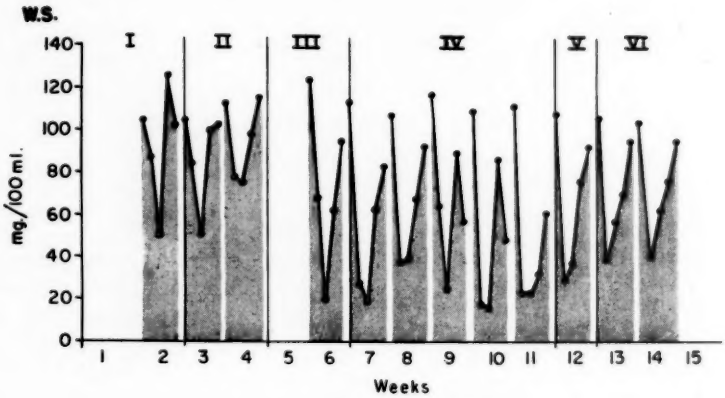

Fig. 8. Insulin sensitivity test.

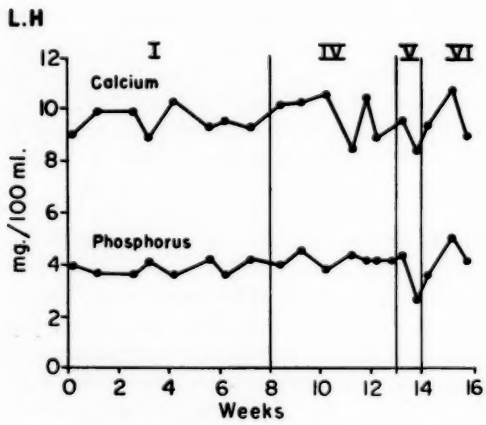

Fig. 9. Calcium and phosphorus.

Serial determinations of serum calcium and phosphorus failed to reveal any alteration (Fig. 9). W. S. had a slight elevation of alkaline phosphatase during the latter half of Period IV, which persisted during V and VI. Serial blood urea nitrogen determinations revealed no alterations from the control values, nor did repeated sodium determinations. Alterations in the serum potassium, serum chloride and carbon dioxide combining power were of striking magnitude (Figs. 10 and 11). In subject H. T. a trend toward the development of hypochloremic alkalosis with hypokalemia began during the first week of Period IV. In subjects W. S. and L. H. extreme degrees of hypochloremic alkalosis and hypokalemia

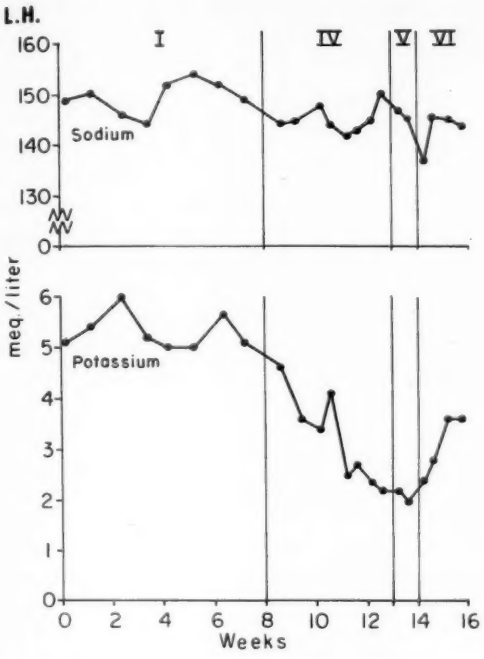

Fig. 10. Serum sodium and potassium.

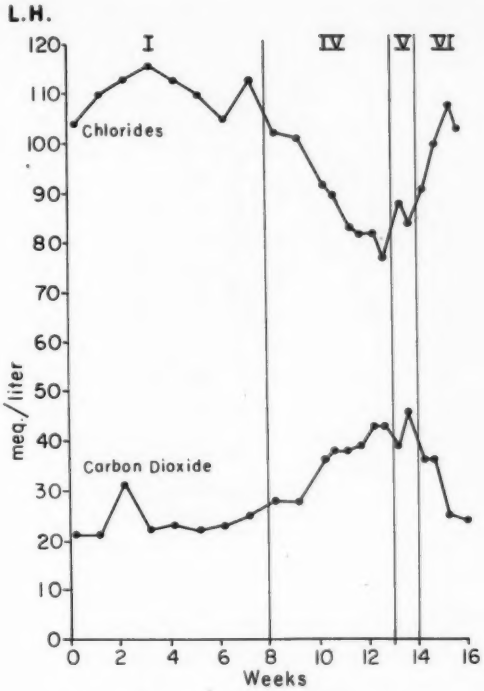

Fig. 11. Serum chlorides and carbon dioxide.

developed during Periods III, IV, and V, with a rapid return to normal occurring during Period VI.

Serum Protein Studies

In all subjects the serum albumin and total proteins remained normal. There were definite alterations in the electrophoretic pattern, with an increase in the alpha-I and alpha-II globulins during the deficient periods in all three subjects, with a return toward control levels during Period VI. In L. H. there was also a definite decrease in the beta-globulin fraction during Period IV. No significant alterations in gamma-globulin were noted (Fig. 12).

Liver Function Tests

Liver function studies, including the bromsulphalein test, thymol turbidity, cephalin flocculation, zinc sulfate turbidity, and serum bilirubin determinations, were performed every two weeks on subject W. S. No alterations were noted.

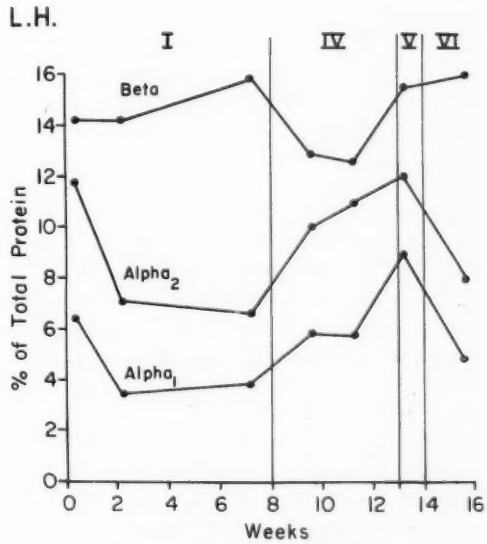

Fig. 12. Plasma proteins (electrophoresis): plasma globulin fraction.

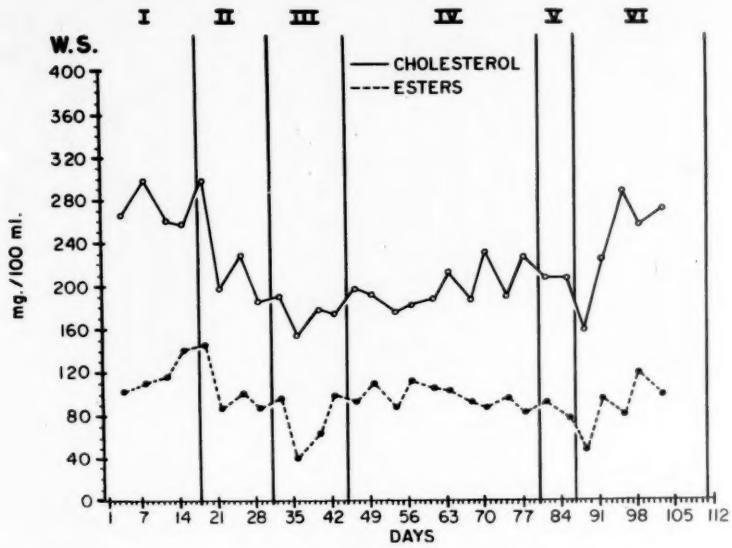

Fig. 13. Cholesterol and esters.

Cholesterol and Cholesterol Esters

Figure 13 gives a representative curve for the changes in cholesterol and esters. There was a sharp fall in both components while the tube feedings were used, with a return to normal when the normal diet was restored. Total serum fat and phospholipids showed no significant changes.

Prothrombin Activity

A ratio of the control prothrombin time to the patients' prothrombin time times 10 was arbitrarily selected as a measure of prothrombin activity. Using this ratio, there was a definite decrease in prothrombin activity during the deficient periods in subject W. S., with a rather prompt return to control levels during Period VI. A similar but somewhat less marked decrease in prothrombin activity occurred in subject L. H. (Fig. 14).

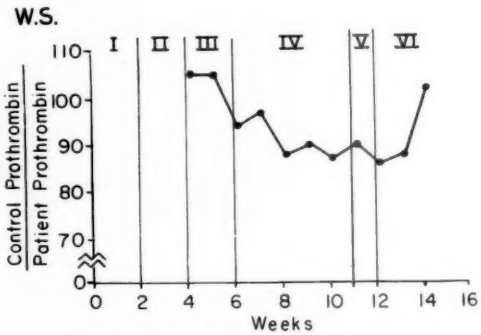

Fig. 14. Prothrombin time.

DISCUSSION

In our previous studies, we were impressed by the clinical illness which occurred when the deficient diet and ω-methylpantothenic acid were used together. In the present study there was a similar weakness, fatigue, and decrease in spontaneous activity. Mood changes, though they did develop, were not as impressive as those observed in previous tests. Dizziness and unsteadiness were severe. Sometimes changes in position were awkward because of the temporary instability. At least some of the fatigue, weakness, and awkwardness seemed to be caused by the neuromuscular disorder. It is impossible to say whether mental disturbances were responsible for one subject leaving the hospital without permission, but such behavior is hardly surprising under the circumstances of the test. One subject developed a psychosis with paranoid features which continued in Period V but rapidly vanished during Period VI. The

torpor and somnolence began to diminish very soon after the antagonist was stopped and pantothenic acid was given in Period V, suggesting strongly that the induced state of pantothenic acid deficiency was responsible for such changes. The neuromuscular abnormalities consisting of paresthesias, weakness, cramps, tenderness and alteration in tendon reflexes, muscle tenderness, and the positive Trousseau sign did not all clear up until Period VI, but there was a very definite improvement in Period V when pantothenic acid was substituted for the antagonist.

The subjects in the present test did not exhibit much vascular instability, postural hypotension, labile pulse, or easily provoked tachycardia, all of which had been so prominent in earlier studies. The ballistocardiograms became abnormal in one subject. Having been abnormal in the control period of another subject, they changed toward normal. Such observations do nothing to clarify the nature of the circulatory status of the subjects, or the vagaries of the ballistocardiogram.

There was no tendency for the subjects to have infection, though the conditions of metabolic ward and of the entire experiment were unchanged from previous tests when infection prevailed during the period of deficiency. There was, however, a striking increase in sedimentation rate, which returned to normal in Period VI. The reason for the increased speed of sedimentation is not known.

Several metabolic changes were studied in detail. In subject W. S., the eosinopenic response to ACTH did not decline until Period IV when the antagonist was given. By the third week of the deficient period, the response had diminished conspicuously. In contrast to our previous observation, this did not come back to normal during the recovery period. The failure of restoration to normal when pantothenic acid was given and when the diet was normal may mean that the mechanism responsible was disturbed more seriously than in the earlier studies when the deficiency-and-antagonist period was 4 rather than 5 weeks in duration. Another possibility is that cortisone, which was used at the termination of the experimental period in the earlier test, had some other effect on the adrenals. The capacity of one subject to excrete a normal amount of urine in response to an ingestion of a large quantity of water was seriously impaired during the deficiency period, but came back to normal during Period VI.

The excretion of 17-ketosteroids is recorded in Figure 6. In one subject the initial levels were higher than normal and fell very promptly while PABA was given. They then returned to the previous level when it was discontinued. Subsequent administration was followed by a diminution of the ratio and then a tendency to level off at the previous control level. During the period of induced deficiency, there was a slow but rather steady decline, which was still continuing at the time the last test was done during the recovery period. The significance of these findings is uncertain.

The changes in glucose tolerance were characterized by prolonged elevation of the blood sugar, which even after two hours tended to stay up around 200 mg per 100 ml or higher, particularly during the latter part of Period IV. This returned to normal during Period V and remained normal. Study of the insulin tolerance test revealed that with the deficient diet alone a sharp increase of the insulin sensitivity occurred; and this persisted throughout Period IV. It was slowly being restored toward normal in Periods V and VI.

Figure 10 shows the very significant fall in serum potassium and the relative stability of the level of serum sodium throughout the experiment. These changes in serum potassium occurred during a period when the intake of potassium was constant. Figure 11 shows the striking changes in blood chlorides and carbon dioxide. Unfortunately we do not have data from balance studies, so we cannot say whether there was a potassium diuresis, an absorption defect, or whether potassium was stored in the body. The electrocardiographic changes indicate that there was indeed a cellular depletion of potassium.

Consistent, but not very extensive, declines in prothrombin occurred during the deficient period, but there was no sign of any liver malfunction nor was clinical bleeding a problem at any time.

SPECULATION

This report deals with work in progress. We are slowly improving the experimental design which we hope eventually will enable us to understand the nature of the changes we have produced. So far we cannot be sure (1) that we have produced a defect in pantothenic acid metabolism by employing a metabolic antagonist which interferes with the diverse functions of coenzyme A; (2) whether ω-methylpantothenic acid is a more powerful toxic agent working as a general protoplasmic poison; or (3) whether some unrecognized deficiency exists in the experimental diet.

As far as the first point is concerned, many of the induced changes are similar to those induced in animals by pantothenic acid deficiency. Perhaps too much emphasis should not be put on the fact that glucose tolerance returned to control levels in Period V without a change in diet but when pantothenic acid replaced the antagonist (Fig. 7). Many other abnormalities did not disappear until Period VI. In retrospect, Period V was too short. We must emphasize the well-known fact that correcting a specific deficiency in a diet does not necessarily correct a lesion induced by the deficiency. Too little, too brief, or too late may explain failures in correcting what has been called the humpty-dumpty situation.[4] Final proof will depend on our ability to titrate the human deficiency syndrome, if such it is, to the stage where it is still quickly reversible by merely replacing the antagonist with the vitamin. The interpretation of any vitamin antagonist's action must be made with knowledge that many subtle metabolic bypasses may enable the cellular and humoral economy to make remarkable adjustments.

Not knowing, except by inference and extrapolation, what coenzyme A and pantothenic acid do in human metabolism, interpretation of our data, at the present merely tentative stage of work in progress, is impossible. If one is willing to compare our observations with a miscellany of observations in a variety of animals, and some of man, it may be that potassium deficiency alone is adequate to explain many of the clinical and biochemical changes.[5–7] Our next tests will inquire into that possibility. We propose to study the effects of large doses of potassium at a time when the serum level is low. Likewise, administration of coenzyme A may tell us whether that substance can correct the metabolic errors. The possibility that our metabolic mischief is related to stimulation of aldosterone production and reduced production of cortisone-like compounds is a stimulating speculation.

If we are dealing with point (3), an unrecognized deficiency, a longer Period V should provide the clue.

CONCLUSION

Work on a syndrome induced in normal subjects, and one with evidence of adrenal cortical overactivity, confirms and extends our observations. A clinical state of lethargy, weakness, burning paresthesias, and cramps with signs of tetany was observed. Low serum potassium may account for many of the clinical findings. Likewise, hypokalemia, hypochloremia, hypochlorhydria, metabolic alkalosis, and a defect in carbohydrate metabolism were observed. Further studies are in progress to elucidate the mechanisms whose disordered functions lead to these disorders.

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DISCUSSION

DR. B. T. TOWERY (Nashville, Tenn.): At the time Dr. Darby asked me to comment on Dr. Bean's paper I was not completely familiar with the experimental evidence which implicated the adrenal cortex in pantothenic acid deficiency. Nevertheless, there are some data which suggest that pantothenic acid deficiency in rodents leads to suppression of adrenocortical function, and it is of interest that Dr. Bean's initial report (*J. Clin. Investigation* 34: 1073, 1955) suggested that the human adrenal responded in a similar manner. However, in attempting to correlate this evidence with the unpublished studies which he has just reported one encounters some intriguing problems.

In view of the character of the response to ingested glucose, one would not anticipate an abnormal sensitivity to insulin, yet such was the case. If one attempts to explain these divergent findings in terms of alterations in adrenocortical function, the difficulty is readily apparent, since hyperglycemia suggests hyperadrenocorticism and marked sensitivity to insulin is a notable feature of Addison's disease. If the latter abnormality is disregarded, the remaining evidence suggests an augmentation of adrenocortical function, because hypokalemic alkalosis was apparently the most striking abnormality noted. You will recall that this electrolyte disturbance is seen rather frequently in association with Cushing's syndrome. The observations of Conn indicate that hyperadrenocorticism with respect to aldosterone alone results in severe potassium depletion; aldosterone excess may, in part at least, account for the hypokalemic alkalosis of Cushing's syndrome. This would not, however, explain similar electrolyte changes which may occur during cortisone therapy.

Dr. Bean's most recent evidence suggests, therefore, that pantothenic acid deficiency in humans may lead to alterations in the pattern of adrenal cortical steroid secretion, so that hydroxycorticoids or, perhaps, aldosterone assume a greater share of net adrenal function.

The insulin sensitivity which was observed, however, is not in accord with such an explanation but suggests other possibilities, for example, hepatic dysfunction with respect to the storage of glycogen and its hydrolysis. It is entirely possible that this response to insulin is a crucial observation because it implies that the adrenal may not be primarily involved in the changes already noted. Dr. Bean has noted briefly some of the lines of research which appear to offer promise. Obviously, further conjecture regarding adrenal function is not warranted until the exact nature of the potassium depletion is elucidated. Should pantothenic acid deficiency lead to abnormalities of potassium absorption or exert direct effects upon the renal excretion of potassium, our preoccupation with adrenal cortical phenomena may prove to be unfounded.

I should like to inquire regarding the route of administration of the ACTH which was used to test adrenocortical responsiveness. Certain ACTH preparations exhibit surprisingly little activity after intrasmuscular injection, yet are quite potent when given intravenously. I'm sure that one must take this fact into account in the interpretation of such studies.

Finally, it is apparent that the work which has been reported serves to emphasize our lack of insight into the mechanism of action of vitamin antagonists and poses many new problems for future study.

DR. F. S. DAFT (Bethesda, Md.): First, I should like to pay my compliments to Dr. Bean for entering this very difficult field and apparently bringing pantothenic acid deficiency in the human somewhat past the embryonic stage. It is very true that there are certain things that we would like to see done before we are completely convinced that this is pantothenic acid deficiency *per se*. It would be very nice, indeed, if we could produce pantothenic acid deficiency in the human merely by keeping the vitamin out of the diet. This is, of course, a difficult thing to accomplish. The fact that the signs which were produced in the human were not completely reversed by pantothenic acid doesn't seem to be necessarily incompatible with this being a pantothenic

acid deficiency, since we have other parallel situations—with folic acid antagonists for example. The signs produced with folic acid antagonists are very difficult to reverse with folic acid, while some derivatives of folic acid, such as citrovorum factor, are much more effective. It is possible that in the present case pantethine or coenzyme A might be more effective than pantothenic acid itself in reversing the deficiency state. I think that all of us would agree that you are on the right track in the sense that pantothenic acid must be a metabolic necessity for the human as well as for all experimental animals.

Despite the lateness of the hour I should like to take just a moment here to mention some work we have been doing in the past several years on pantothenic acid deficiency in rats. As you know, panthothenic acid is considered to be a dietary essential for the rat. Yet, under certain circumstances, we have been able to raise rats from weaning up to the level of 500 or 600 grams while they are receiving no pantothenic acid in the diet. The methods that we have used have been three in number. One was the inclusion of a large amount of vitamin C in the diet, the second was the use of antibiotics in the diet, and more recently we have used a very interesting compound prepared by Smith, Kline and French which is known as compound 525A. The animals have been continued on these diets for long periods of time—a year or longer in some cases—and it has thus been shown that rats can grow to maturity with no pantothenic acid in the diet, or rather with the very small amount which is found in "vitamin-free" casein. Here, we would say very definitely, knowing what we do about the functions of coenzyme A, that despite the fact that pantothenic acid is not under these conditions a *dietary* essential, it is certainly a *metabolic* essential. We have studied this point by determining the total pantothenic acid in the body of weanlings and in littermates kept for several weeks on a diet virtually free of pantothenic acid but containing large amounts of vitamin C. We have shown that these animals contain approximately 20 micrograms a day of pantothenic acid in excess of that with which they started. Therefore, this is not a case of an animal surviving and growing without pantothenic acid but instead a situation where pantothenic acid is being manufactured somewhere in the body. As yet we haven't determined the site of manufacture. Most people will jump to the conclusion that the pantothenic acid is being furnished by intestinal bacteria. This is possibly true, but we are far from completely convinced that this is the whole story.

There is one aspect of the work with humans which I would like very much to see followed a little further. As you know, whether or not pantothenic acid deficiency truly produces a lack of adrenal hormones in experimental animals is still an open question to a certain extent. Most of the evidence which has been presented from time to time suggests that during pantothenic acid deficiency there is an adrenal insufficiency. One bit of evidence, however, is very hard to fit in with this idea. You will remember that when Dr. Elaine Ralli, using black rats which had turned gray during pantothenic acid deficiency, removed the adrenals of these animals, their hair turned black again. Until those experiments were reported I believe that most investigators in the field had felt certain that pantothenic acid deficiency led to a deficiency of adrenal steroids. Here is a case, however, where stopping almost completely the production of adrenal steroids (by adrenalectomy) reversed the effect of pantothenic acid deficiency. The effect of adrenalectomy could be reversed, in turn, by the administration of adrenal steroids, showing that these substances were in fact involved in this phenomenon. I remember in your paper, Dr. Bean, that you did some studies of steroid excretion in the urine. I should think it would be most interesting, indeed, to find out something more about the quantitative output of individual steroids. That is a project which we have had in mind for a long time in conjunction with our studies in rats. The technical difficulties of making these determinations on rats is great, but they could be done more readily with human subjects.

DR. W. BEAN (Iowa City, Iowa): I thank the discussants for sharing with me the con-

clusion that I have added more confusion than solutions for specific questions. The ACTH was given intramuscularly. I am sure I do not understand how we should interpret the rather paradoxical behavior of the glucose tolerance test and the increased sensitivity to insulin. A number of ready interpretations can be brought forth, but we have no real notion which is right, or indeed, whether any is right, and this is one of the problems to which we are addressing our attention in the future. We have not included many studies on steroid hormones, which obviously should be done. In experiments now in progress, we are trying to do a balance study on potassium, and we will assay the urine for several of the steroid hormones. We cannot emphasize too strongly the fact that this is work in progress and is a long way from a definitive exposition of the many problems we are uncovering in this approach to a better understanding of vitamin function.

Effect of an Elixir on the Absorption of Vitamin B_{12} by Healthy Young and Old Subjects*

By BACON F. CHOW, PH.D., ANDREW HORONICK, B.S., AND KUNIO OKUDA, M.D.

THE ABSORPTION of orally administered vitamin B_{12}, even by healthy people, is poor.[1] In patients suffering from pernicious anemia,[2,3] or in those with complete gastrectomy,[4] vitamin B_{12} absorption is negligible. The oral coadministration of normal gastric juice or intrinsic factor preparations can increase the vitamin B_{12} absorption in the latter groups; however, whether such agents can also increase absorption in clinically healthy subjects has not yet been demonstrated by any systematic study. On the contrary, it has been found that an excessive dose of the usual intrinsic factor preparations may inhibit the absorption of vitamin B_{12} by normal rats,[5] by young, healthy humans,[6] and by gastrectomized subjects.[4] However, such data need not support the hypothesis that intrinsic factor, *per se*, is inhibitory. It has been demonstrated that highly purified preparations, or those prepared from sources without inhibitory substances, may actually enhance absorption.[6] This paper reports on several experiments designed to evaluate the ability of an elixir containing vitamin B_{12}, other vitamins, and lipotropic substances† (hereinafter referred to as the "elixir" with vitamin B_{12}) to enhance vitamin B_{12} absorption in both normal subjects and those suffering from pernicious anemia.

From the Department of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md., and The Institute of Geriatrics of the Daughters of Jacob, Bronx, N. Y.

The authors acknowledge with thanks the grants-in-aid and material supplies from Smith, Kline & French Laboratories, Atomic Energy Commission No. AT-30-1203, The National Vitamin Foundation, Merck & Company, Inc., and the Lederle Laboratories.

* Not presented at the Symposium.

† Liptril®, Smith, Kline & French Laboratories, Philadelphia, Pa.

METHOD

Medications Used

The following medications were used in these studies: commercial vitamin capsules containing all known vitamins and minerals except vitamin B_{12},‡ capsules containing 100 μg of vitamin B_{12}; solutions containing either 50 μg of radioactive (Co^{60}) vitamin B_{12} or 1000 μg of regular vitamin B_{12}; and the "elixir" containing vitamin B_{12}. One teaspoonful (5 ml) of the "elixir" with vitamin B_{12} contains the following ingredients: vitamin B_{12} (crystalline) 8.34 μg; riboflavin, 0.6 mg; niacinamide, 7.0 mg; pyridoxine, 2.0 mg; betaine, anhydrous, 700.0 mg; choline dihydrogen citrate, 150.0 mg; inositol, 150.0 mg; ferric pyrophosphate, 35.0 mg; caffeine citrate, 1 grain (65 mg); and alcohol, 15 per cent.

I. Studies of Vitamin B_{12} Serum Levels

A preliminary study was conducted in an old people's home by assaying the serum vitamin B_{12} levels of 21 elderly patients (65 years or older) who had been receiving the "elixir" with vitamin B_{12} (one teaspoon three times a day) for three months, and comparing the vitamin B_{12} serum levels of these patients with those of 139 patients who had not received this treatment (Fig. 1).

The results of the preliminary study prompted us to conduct a second one on 60 healthy patients at a different home for the

‡ Gevral, Lederle Laboratories, Pearl River, N. Y.

aged. All of the patients chosen had vitamin B_{12} serum levels of less than 175 $\mu\mu$g per ml. They were randomly divided into two groups of 30 patients each. Group A received a daily

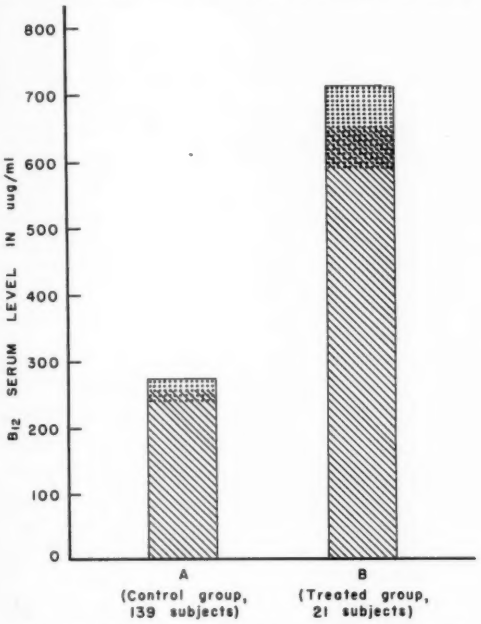

Fig. 1. Results of first serum level study. The cross-hatching in the columns denotes the magnitude of two standard deviations. The dividing line between the cross-hatching and the dotted portion immediately above it is the average value of the vitamin B_{12} serum level.

capsule containing all known vitamins and minerals *except* vitamin B_{12}; Group B received a capsule containing 100 μg of vitamin B_{12}, in addition to the capsule received by Group A. Serum levels[7] were determined at frequent intervals for seven months. At the end of this period all but 16 patients (8 from Group A and 8 from Group B) were dropped from the experiment because they had either failed to follow the regimen faithfully, or because they were unwilling to submit to the laboratory procedures. At the beginning of the eighth month, four of the eight patients remaining in Group B were given 15 ml of the "elixir" with vitamin B_{12} once a day (equal to a daily vitamin B_{12} dose; 25 μg). The remaining four patients continued to receive 100 μg of vitamin B_{12} a day. The blood serum levels of all groups were compared (Fig. 2).

A third study was then conducted on another 40 elderly patients with vitamin B_{12}

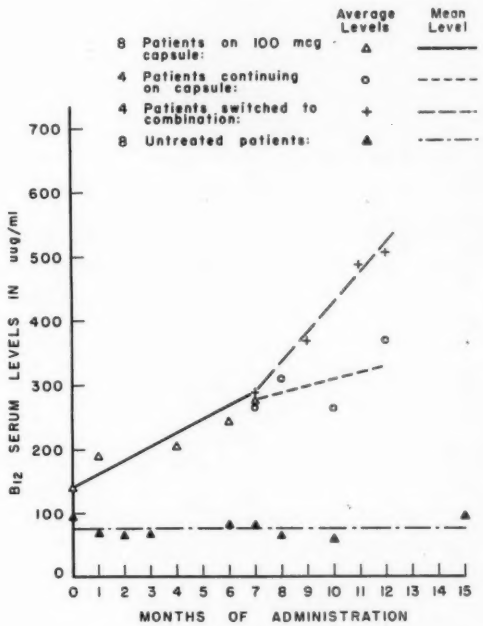

Fig. 2. Results of second serum level study.

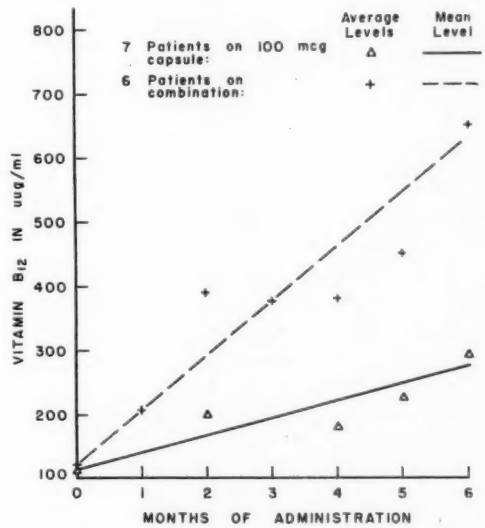

Fig. 3. Results of third serum level study.

serum levels of less than 175 μμg per ml. Once daily, 20 of them (Group A) received a capsule containing 100 μg of vitamin B_{12}. The other 20 (Group B) received 15 ml of the "elixir" with vitamin B_{12} (daily B_{12} dose; 25 μg). Blood specimens were taken at frequent intervals. After four months of treatment all but 13 patients (7 from Group A; 6 from Group B) had dropped from the experiment. A comparison of results in these 13 patients is given in Figure 3.

II. Urinary Excretion Studies

Method of Assay: The complete details of the urinary excretion test are published elsewhere.[8] Briefly, the method was as follows: 10 ml of an aqueous solution of 50 μg of radioactive vitamin B_{12} (specific activity, 14.4 μc/mg) were mixed with either 10 ml of water or 10 ml of the "elixir"; this "elixir" contained all the components of the "elixir" with vitamin B_{12} except vitamin B_{12}. Precisely two hours after taking one or the other solution, each patient received 1 ml of a vitamin B_{12} solution (1000 μg/ml) intramuscularly. A 24-hour urine specimen was collected. (At first, both 24- and 48-hour specimens were collected, but it was found that the 48-hour specimen yielded less than 5 per cent of the vitamin B_{12} excreted during the first 24 hours.)

Each specimen was measured by pouring one half of the urine into a beaker containing 50 μg of unlabeled vitamin B_{12} as carrier. After evaporation on a steam bath, the contents of the beaker were transferred into a bottle graduated to 50 ml and made up to volume. The bottle was then inserted into the well of a scintillation counter for radioactivity measurement.

Study Procedure: The study was conducted over an eight-month period on 60 young, healthy volunteers (ages 22–42) from a penal institution. Four separate trials were made in order to compare the urinary excretion of radioactivity in a number of patients who received radioactive vitamin B_{12} plus water, with an equal number who received radioactive vitamin B_{12} with the "elixir" (Table I).

In addition, 15 volunteers received both regimens (Table II); 7 of these (Group A) began on radioactive vitamin B_{12} plus water and were switched to radioactive vitamin B_{12} plus the "elixir"; 8 (Group B) began on radioactive vitamin B_{12} plus the "elixir" and were then switched to the radioactive vitamin plus water.

Urinary excretion tests were also performed in six subjects who had pernicious anemia in remission (Table III). They were given oral doses of 2 μg of radioactive vitamin B_{12} in

TABLE I

The Effect of Administration of an Elixir on the Urinary Excretion of Orally Administered Radioactive Vitamin B_{12} in Young, Healthy Adults

Study	Average radioactive vitamin B_{12} in 24-hr urine samples (mμg)		Statistical significance of the differences
	Controls (25)	Treated group (35)	
I	450 (7)	878 (6)	$P < 0.05$
II	470 (7)	951 (5)	$P < 0.05$
III	529 (7)	622 (6)	$P < 0.05$
		753 (6)	
IV	546 (4)	765 (6)	$P < 0.05$
		670 (6)	

( ) indicates number of subjects studied.

TABLE II

The Urinary Excretion Test on Radioactive Vitamin B_{12} with or without an Elixir by Switchback Experiment

Group	Therapy	Urinary excretion	Difference between (1) and (2)
		mμg in 24 hrs	
A (7)	1. 50 μg + water	706 ± 168	
	2. 50 μg + elixir	1680 ± 110	$P \ll 0.05$
B (8)	1. 50 μg + elixir	778 ± 49	
	2. 50 μg + water	575 ± 90	$P < 0.05$

( ) indicates number of subjects studied.

TABLE III

The Effect of an Elixir on Urinary Excretion of Orally Administered Radioactive Vitamin B_{12} in Patients with Pernicious Anemia in Remission

Subject	Treatment*	
	2 μg vitamin B_{12}	2 μg vitamin B_{12} +30 ml Elixir
A	0.80	1.4
B	0.60	3.0
C	—	0.97
D	0.92	4.1
E	0.8	2.6
F	—	0.9

* Urinary excretion expressed as per cent of the orally administered radioactive vitamin B_{12}.

30 ml of the "elixir," and then received 1.0 mg of unlabeled vitamin B_{12} two hours later by injection.

RESULTS

Serum Level Studies: The results of the first study (Fig. 1) show that the mean vitamin B_{12} serum level in the 21 patients receiving the "elixir" with vitamin B_{12} was 652 ± 61.7 $\mu\mu$g/ml. In 139 patients who did not receive the "elixir" with vitamin B_{12}, the mean serum level was 255 ± 15.8 $\mu\mu$g/ml. This difference is statistically significant.

It should be pointed out that several patients in the control group took the "elixir" with vitamin B_{12} at irregular intervals.* This fact, however, would only tend to increase the vitamin B_{12} serum level of the control group. Undoubtedly, then, the effect of the "elixir" on the vitamin B_{12} serum level was marked.

The results of the second study (Fig. 2) demonstrate that it required from six to eight months to replete tissue reserves by the daily oral administration of 100 μg of vitamin B_{12}. The mean serum level climbed from 100 to 250 $\mu\mu$g/ml. The four subjects (circles) who were continued on a 100-μg vitamin B_{12} capsule for two more months experienced only a slight elevation (from 250 to 369 $\mu\mu$g/ml). On the other hand, the four patients (crosses) who were switched from 100 μg of vitamin B_{12} alone to 25 μg of vitamin B_{12} in the "elixir" experienced a marked increase in serum levels (from 250 to 505 $\mu\mu$g/ml).

The results of the third experiment (Fig. 3) again demonstrated the superiority of the "elixir" with vitamin B_{12} (crosses) to vitamin B_{12} alone (triangles), as determined by serum levels. The average serum level of those on the "elixir" was 630; of those on vitamin B_{12} alone, 275 $\mu\mu$g/ml.

The results of four separate trials on 60 young volunteers (Table I) demonstrate that each subject receiving the "elixir" excreted significantly greater amounts of vitamin B_{12} than those who received vitamin B_{12} alone. Every patient who received both regimens excreted more vitamin B_{12} when taking the "elixir" (Table II). There was, however, considerable variation in the amounts excreted from experiment to experiment.

The results in patients with pernicious anemia (Table III) demonstrate that the "elixir" with vitamin B_{12} was of questionable value in increasing their vitamin B_{12} absorption. Of the 6 patients in remission, 3 had a partial response only; 3 had a negligible response. Of 3 patients in relapse, 2 responded, but the third did not. However, the latter patient had also failed to respond to any other oral therapy.

DISCUSSION

Vitamin B_{12} is essential for human nutrition[9,10] and for the treatment of certain diseases. Its use may be broadened as its importance in treating many metabolic disorders becomes better understood; therefore, it is of the utmost importance to find a means of assuring absorption when this vitamin is given orally. Although it is logical to assume that the oral coadministration of intrinsic factor preparations will increase the absorption of vitamin B_{12} by healthy individuals, data to support this hypothesis are inconsistent and meager. According to some reports[4,5,6] the use of certain intrinsic factor preparations is contraindicated in individuals without achylia gastrica. In our laboratory we have been interested in obtaining simple chemical substances capable of enhancing the absorption of vitamin B_{12}.

It is of particular interest to note that the "elixir" reported on in this paper, which possesses a marked ability to increase vitamin B_{12} absorption, is not derived or extracted from the usual animal sources of intrinsic factor. While differences in excretion were demonstrable in each test, the variation among the test subjects was great. This variation is primarily a reflection of our lack of precise knowledge of the mode of action (be it physical or chemical). It is conceivable that the ability of the combination to enhance vitamin B_{12} absorption may be due to its physical characteristics; to

* In an unknown number of instances, subjects in this control group requested this mixture; hence, the average value of the vitamin B_{12} serum level in the control group is higher than the mean value of serum obtained from other populations.

some chemical ingredients; or even to a chemical reaction which alters the chemical structure of vitamin B_{12} to a form[11] more easily absorbed than is cyanocobalamin.

Intrinsic factor is defined by Castle and associates[12] as a substance present in gastric juice which, when given with vitamin B_{12} by mouth, will produce a therapeutic response in pernicious anemia patients. Subsequent study by Schilling[3] showed that it will increase the absorption of vitamin B_{12} in such patients, according to the urinary excretion test. Thus, the "elixir" used in our study cannot be classified as an intrinsic factor-like substance unless it can regularly enhance absorption in patients with pernicious anemia. Because of its failure to regularly or markedly increase absorption in patients with pernicious anemia, it is questionable whether this combination itself can be considered to possess intrinsic factor activity. Whether the addition of other substances, such as those which bind vitamin B_{12}—a property believed by some to be inherent and essential to intrinsic factor activity—will result in a mixture with true intrinsic factor properties, remains to be answered by further experiments.

SUMMARY AND CONCLUSIONS

In order to evaluate the ability of an elixir containing vitamin B_{12}, other vitamins, and lipotropic substances to enhance the absorption of vitamin B_{12}, several studies were performed. They compared the serum vitamin B_{12} levels of subjects receiving the elixir with those receiving vitamin B_{12} alone. These studies involved 258 patients (189 old people, 60 young normal volunteers, and 9 patients with pernicious anemia). In the elderly people, vitamin B_{12} absorption was evaluated by assays of blood serum; in the volunteers and patients with pernicious anemia, by urinary excretion studies of radioactive vitamin B_{12}.

The results of every study on healthy subjects revealed that the elixir was capable of producing significantly higher serum vitamin B_{12} levels than could be obtained with larger doses of vitamin B_{12} alone. The elixir was of questionable value in treating patients with pernicious anemia.

It is concluded that this elixir is a singularly effective agent for treating vitamin B_{12} deficiency not caused by deficiency of intrinsic factor; that it enhances the absorption of B_{12}; and that the preparation cannot as yet be considered to have intrinsic factor properties.

ACKNOWLEDGMENT

The authors express their appreciation for the faithful services of Miss Alicia Willer.

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Vitamin B_{12} Serum Level and Pregnancy*

By KUNIO OKUDA, M.D., ANDRE E. HELLIGER, M.D., AND BACON F. CHOW, PH.D.

VITAMIN B_{12} is one of the essential factors in human nutrition[1] and in the maintenance of normal health.[2] It is, therefore, of interest to study the degree of tissue saturation with this vitamin in pregnant women and in their offspring, as measured by maternal and fetal serum vitamin B_{12} levels at the time of delivery. The results of such a study are presented in this communication.

EXPERIMENTAL

Vitamin B_{12} Assay: The procedure[3] for the determination of vitamin B_{12} activity in serum may be described briefly as follows: 2 ml of serum are added to a tube containing 8.0 ml of acetate buffer, pH 4.6. The tube is covered snugly with a piece of tinfoil and heated in a large boiling water bath for 30 minutes. After cooling with cold water, the tubes are centrifuged for 5 minutes and 6 ml of the clear supernatant fluid are removed and neutralized with 1 ml of Na_2HPO_4 (0.33 molar). 1.0, 2.0, and 3.0 ml of this mixture are then measured and assayed for the microbiological activity of vitamin B_{12} with the procedure of Skeggs and Wright.[4] The alkali-stable factor in the serum was determined by the procedure of Hoffman[5] *et al.*

RESULTS

Blood specimens for the vitamin B_{12} assay were obtained by venipuncture from the veins of a series of 25 pregnant women at the time of delivery and from the umbilical cords of their infants. The results of the assays, the ages of the mother and the weights of the infants at birth are shown in Table I. The ages of the women ranged from 15 to 41 years, with an average of 28 years. Parity of the women ranged from zero to eight. Approximately one-half of the subjects were white, and the other half were colored. The mean vitamin B_{12} serum level of the fetus was considerably higher than that of the mother, being 358 ± 36 and 120 ± 14 $\mu\mu$g/ml, respectively. The difference is statistically significant ($P<0.001$).

For comparison, blood specimens were drawn for vitamin B_{12} assay from non-pregnant women of comparable age; these subjects were employees of the Johns Hopkins Hospital and the School of Hygiene. The average vitamin B_{12} serum level of this group was 185 ± 12 $\mu\mu$g/ml. This was a selected group of women and may not be strictly comparable with our study group.

In a separate study, we recently measured the vitamin B_{12} serum level of another group of young, clinically healthy, non-pregnant women, selected at random from the Baltimore population.[4] The mean serum vitamin B_{12} value of this group was 220 ± 21 $\mu\mu$g/ml. Thus the serum levels of both groups of non-pregnant subjects were significantly higher than those of the pregnant women at the time of delivery.

In Figure 1 the vitamin B_{12} serum values of mothers are plotted as abscissa against those of the cord serum as ordinate. At a 45° angle a theoretical line is drawn on which points would fall if the maternal and cord values were equal. The points would be below or above this line, if the maternal values were larger or smaller than the fetal values. In every one of the 25 cases studied the cord level was at least equal to, or in excess of, that of the mother. There was no detectable amount of vitamin B_{12} activity in cord serum extract after alkali treatment; therefore, the higher vitamin B_{12} value in fetal serum is not due to desoxyriboside. The probability that all dif-

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*Not presented at the Symposium.

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TABLE I

Serum B_{12} Levels of Mother and Fetus

Patient	Age	Race	Para	Mother, serum B_{12}	Fetus, cord serum	Wt. of newborn
				μμg/ml	*μμg/ml*	*g*
1	20	C	III	114	333	2560
2	15	C	0	247	925	2805
3	37	C	I	87	281	2880
4	21	W	IV	119	128	3620
5*	19	W	II	<40	65	3960
6	22	W	I	230	<A270	3400
					<B330	3200
7	21			163	350	
8	26	C	I	117	280	2970
9	28	W	I	170	385	3840
10	41	C	III	~64	~262	2980
11	24	C	II	93	216	3950
12	32	W	III	105	420	4600
13	23	C	0	160	400	2878
14	27	W	IV	106	~410	
15	25	C	I	~40	347	3055
16	18	C	I	72	310	2615
17	28	W	0	319	435	3125
18	27	C	0	160	595	3750
19	21	C	0	115	275	2270
20	25	W	I	~53	178	3425
21	29	C	I	150	444	3900
22	29	C	I	<15	667	3050
23	35	W	IV	~90	178	3300
24	28	C	II	101	295	3075
25	36	C	VIII	84	461	3170
				119.8 ± 14.1	357.6 ± 35.8	$t = 4.17$

* Cretinism.

ferences would be of the same numerical sign in a population of 25 is extremely small.

DISCUSSION

Although vitamin B_{12} is considered to be essential for the growth of children, its stimulatory effect cannot be demonstrated easily in clinically healthy full-term[6] or premature infants, but can manifest itself among undernourished children.[6,7] In the light of our results, this difference in response may be due to the variation in the reserve of vitamin B_{12} in the young. The unique property of the fetus to concentrate vitamin B_{12} was reported in our previous communication,[8] where radioactive vitamin B_{12} was injected into pregnant rats about two weeks before parturition. In relation to the body weight, a large portion of the injected radioactivity, taken as a measure of vitamin B_{12}, was found in the fetus. Besides vitamin B_{12}, ascorbic acid[9] is the only other vitamin known to be concentrated in the offspring in a similar manner.

The serum level of vitamins may be considered an index of tissue saturation. Diseases due to vitamin B_{12} deficiency (such as pernicious anemia), or situations in which the dietary intake is inadequate[10] or absorptive capacity poor, will be accompanied by low vitamin B_{12} serum levels. On the other hand, elevated levels of the vitamin may be totally irrelevant to tissue saturation, since in certain diseases, such as leukemia[11] and diabetes with retinopathy,[12] the serum level may be elevated, presumably because of the destruction of tissues to which vitamin B_{12} is bound. It is our belief that a low vitamin B_{12} level reflects poor tissue reserves of this vitamin, although the reverse may not be necessarily true.

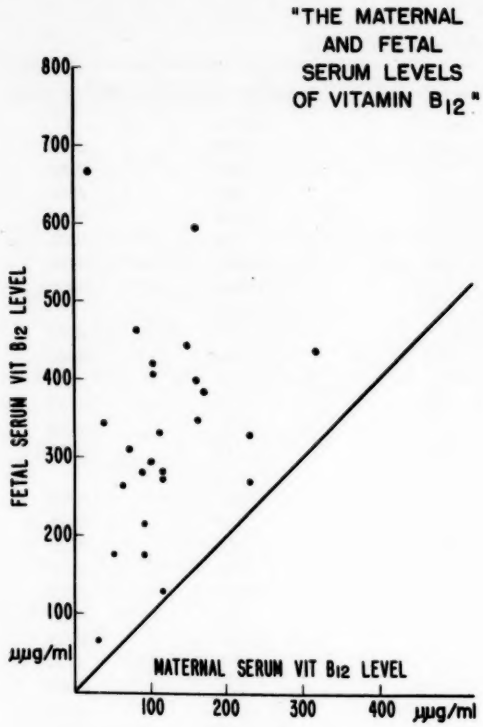

Figure 1

While our results definitely show abnormally low vitamin B_{12} serum levels in the mothers at the time of delivery, any justification for administering this vitamin to pregnant women must be based on the advisability of repleting the vitamin B_{12} level of the mother and insuring an adequate supply to the fetus. No clinical data are yet available to justify the general use of this vitamin in pregnancy.

With the limited number of cases studied, it is not possible to correlate the level of vitamin B_{12} in the cord serum either with the parity of the mothers or with the weight of the newborn infants. It is of interest to record that the vitamin B_{12} level in the cord serum of Case 5 was abnormally low for this series. Seven weeks after delivery this infant was admitted to the pediatrics department of the Johns Hopkins Hospital with marked and classical signs of hypothyroidism and bone growth retardation, as seen in cretinism.

It might be of interest to speculate whether a certain fetal level of vitamin B_{12} is necessary, in certain as yet unspecified stages of intrauterine life, for the proper development of unspecified fetal organs (e.g. the thyroid gland or bone).

SUMMARY

Vitamin B_{12} serum levels of mother and fetus were determined in 25 women ranging in age from 15 to 41 years, and in their first to ninth pregnancy. It was found that the vitamin B_{12} serum level of the maternal blood was considerably lower than that of the nonpregnant women of comparable age, and also lower than that of the fetal cord level. These findings may indicate that vitamin B_{12} is drawn from the mother to the fetus.

ACKNOWLEDGMENT

The authors express their appreciation for the cooperation and assistance of Dr. N. J. Eastman.

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Nutrition News

International Congress of Internal Medicine

The Fourth International Congress of Internal Medicine, organized by the International Society of Internal Medicine, will meet this year in Madrid, September 19–23. After an initial symposium on *The Present Situation of Internal Medicine*, successive meetings will deal with the following scheduled themes: *The Role of the Adrenals in the Pathogenesis and Evolution of Internal Disease* and *Bronchial Asthma and Emphysema*. A final session will be given over to free choice communications. Among the many distinguished speakers are Dr. H. Selye (Canada), Dr. Jerome W. Conn (United States), and Dr. J. Groen (Netherlands), a Corresponding Editor of this JOURNAL.

Food and Drug Anniversary

Public attention was centered on the 50th birthday of federal food, drug and cosmetic legislation by a national "Food and Drug Law Golden Anniversary Week," June 24–30.

Industries in the food, drug, chemical, and cosmetics fields joined with food and drug enforcement officials and consumer groups to hold commemorative events throughout 1956, with special emphasis on the final week of June.

It was on June 30, 1906, that President Theodore Roosevelt signed into law both the original federal Food and Drugs Act and Meat Inspection Act, the foundations of today's legal and administrative operations whereby industry and government work in partnership to provide American consumers with what is considered the world's best food and drug supply.

Highlights of the June week in Washington included day-long golden jubilee ceremonies on June 27 and issuance of a special three-cent postage stamp on the same date.

In addition, governors, state enforcement officials, and industry leaders have begun a series of anniversary events in the states. Women's organizations, recalling the active part played by housewives in sponsoring the original pure food and drug law 50 years ago, are likewise conducting anniversary meetings and activities.

The Association of Food and Drug Officials of the United States, whose 50th Anniversary Committee is sponsoring the nation-wide program of commemorative projects, devoted its annual national conference in New York, May 6–11, to the birthday of the laws.

Marking a half century of progress under what has been termed "the most significant peacetime legislation in our history," industry organizations, professional associations and individual firms in the food, drug, cosmetic and chemical industries are featuring the anniversary in exhibits, speeches, panel discussions and similar activities.

Margaret A. Ohlson

Dr. Margaret A. Ohlson has been appointed professor and head of nutrition at the State University of Iowa Hospitals, effective July 1. Currently professor and head of the food and nutrition department at Michigan State University, Dr. Ohlson will succeed the late Dr. Kate Daum, who was head of nutrition at Iowa from 1926 until her death Dec. 31, 1955. Dr. Ohlson also was named professor of nutrition on the department of internal medicine staff in the University's College of Medicine.

Now president of the International Dietetic Association, Dr. Ohlson is the recipient of both the Borden Award in home economics (1950) and the American Dairy Association Award in research (1953). She also served as consultant in dietetics to the surgeon general of the United States Air Force.

Foreign Chemicals in Foods

The Second Symposium on Foreign Chemicals in Foods, organized by the Netherlands Association for Nutritional Research under the

auspices of the Commission Internationale des Industries Agricoles and the Bureau International Permanent de Chimie Analytique, was held at Amsterdam from the 9th to the 11th of July, 1956.

This symposium was preceded by a meeting, on the 6th and 7th of July, of West European experts, who continued the discussions on foreign chemicals in foodstuffs that were initiated in Bad Godesberg and São Paulo.

The conclusions of these discussions will be submitted to the symposium, which will also discuss the biological integrity of foodstuffs, the influence of industrial processing (refining of staple foods and their improvement through enrichment), and the possibilities of international agreement as regards protection of the nutritive value of foodstuffs. The possibility of composing a European food codex will also be explored.

New England Nutrition Conference

On March 22–23, the state of Florida, through the Florida Citrus Commission, sponsored the New England Conference on Human Nutrition—designed as an experiment in public education for improved nutrition.

Unlike most nutrition conferences, aimed at initiates or, at least, physicians, this one was addressed primarily to "lay opinion leaders," present by invitation and picked to represent business and industry, labor and agriculture, service organization, schools, etc.

Such authorities as Drs. George R. Cowgill, James M. Hundley, S. L. Halpern, and James H. Shaw were on hand to impress on the laity the importance to health of sound nutrition, and the distinguished anthropologist, Dr. M. F. Ashley Montague located nutrition in the broader context of human nature.

It would be ironic if conferences directed at lay leaders were to prove more successful than attempts at nutritional indoctrination by (and of) physicians, and nutritionists will await the results with interest.

Physiological Congress

Physiologists will foregather this year in Brussels, where the Twentieth International Physiological Congress will meet from July 30 to August 4. Among the eight symposia scheduled, two will be of special interest to workers in the field of nutrition: *Physiology of Water and Food Intake* and *Physiology of Acid Secretion by the Stomach*.

Reviews of Recent Books

Progress in the Chemistry of Fats and Other Lipids, Vol. III, by R. T. Holman, W. O. Lundberg, and T. Malkin, Pergamon Press, London and New York, 1955, pp. 475, $10.50.

Publications related to the chemistry of fats have, until recently, been largely restricted to scientific journals which were oriented along agricultural or industrial pursuits. Consequently, the physiologist involved in the resurgence of fat biochemistry, which has been triggered by the newer nutritional implications of lipids in cardiovascular diseases, welcomes any synthesis of knowledge in this field. This, the third in a series, presents a compendium of past and present research in lipids which should be invaluable to teachers and research workers. Certain chapters of this book could be especially useful to those doing clinical research. These include discussions of the Parenteral Administration of Fats, The Biochemistry of Fat-Soluble Vitamins, Aspects of the Intestinal Absorption of Fats and the Metabolism of the Steroid Hormones, by Smith Freeman, Henrik Dam, Sune and Bengt Bergström and Leo T. Samuels, respectively.

Although most of the other chapters in this volume are directed to the attention of the chemist, the information contained therein could well be brought to the attention of the practical physiologist. The growing importance of unsaturated fats in clinical medicine makes this book, especially if used in conjunction with the previous volume, most timely. M. K. HORWITT

Annual Review of Medicine, Vol. 7, edited by David A. Rytand and William Creger, Annual Reviews, Inc., Stanford, Calif., 1956, pp. 611, $7.00.

The "Annual Reviews" have come to occupy a regular place on the library shelves of research workers. Their carefully documented surveys—prepared by active investigators—are especially valuable in obtaining a bird's-eye view of current research in a specific field.

This year's book continues the high standards set by previous issues. Our readers will be especially interested in William J. Darby's review of nutrition, covering some 136 references to the current literature. Among the several topics discussed are: protein malnutrition of kwashiorkor, goiter, fluorides, and clinical evaluation of the nutritional status. The field of medicine is so wide that certain disorders are not covered in any one volume. Thus, the subjects of diabetes mellitus, gout, and steatorrhea, for example, are not covered this year; no doubt they will be adequately reviewed in succeeding volumes.

In addition to the complete author and subject indices for all 25 chapters, there is a useful annotated list of review articles, which this year recorded 893 such articles! This series can again be recommended as a valuable guide to the voluminous mass of medical literature. S. O. W.

Books received for review by the AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

Essays in Biochemistry, edited by S. Graff, John Wiley & Sons, Inc., New York, 1956, pp. 345, $6.50.

Les Vitamines, by H. Thiers, Masson et Cie, Paris, 1956, pp. 626, paper 4.000 fr., bound 4.600 fr.

Symposium on Nutritive Aspects of Preserved Foods, Swedish Institute for Food Preservation Research, Göteberg, 1956, pp. 173, 30:- Sw. Cr.

La Régulation des Processus Métaboliques dans l'Organisme, by Th. Cahn, Presses Universitaires de France, 1956, pp. 681, 3.000 fr.

Transactions of the Fifth Meeting of the International Society of Geographical Pathology (Cancer), S. Karger, A. G., Basel, 1955, pp. 950, Sw. fr. 67.60.

Histamine (Ciba Symposium), edited by G. E. W. Wolstenholme and C. M. O'Connor, Little, Brown and Co., Boston, 1956, pp. 472, $9.00.

Practical Cookery and the Etiquette and Service of the Table, Dept. of Nutr., Sch. of Home Economics, Kansas State Coll. of Agric. and Appl. Sc., John Wiley & Sons, Inc., New York, 1956, pp. 364, $4.00.

The Year Book of Endocrinology (1955–1956 Series), edited by G. S. Gordon, Year Book Publishers, Inc., Chicago, 1956, pp. 367, $6.00.

Abstracts of Current Literature

ARTERIOSCLEROSIS: NUTRITIONAL CONSIDERATIONS

Observations on the levels of serum cholesterol and beta-lipoproteins have been conducted by several investigators in active versus sedentary groups of human subjects. These studies have been prompted by the finding that the incidence of coronary heart disease in men engaged in physically active occupations is apparently lower than in those with inactive jobs in similar socio-economic conditions. The findings indicate that more elevated levels of the presumably atherogenic forms of blood lipids develop postprandially in sedentary individuals.

Serum Cholesterol in Men in Basal and Nonbasal States. A. Keys, J. T. Anderson, and O. Mickelsen. *Science* 123: 29, 1956.

Serum cholesterol determinations are usually made on samples obtained under basal, fasting conditions. This study assessed the significance of these conditions. Determinations on normal adults revealed an average nonbasal (mid-morning) value of 3.8 mg per 100 ml higher than the basal value. This difference was only 1.9 per cent of the mean basal level.

Although eating breakfast led to a small rise in cholesterol levels, this change did not occur when breakfast was followed by moderately vigorous physical work. The highest differences, 15 mg/100 ml, were found in sedentary individuals 7 hours after a breakfast (to which 10 g of cholesterol were added to scrambled eggs). Under the same circumstances, physical activity led to a rise of 9.3 mg/100 ml.

The authors believe these results suggest a reason for part of the difference in susceptibility to coronary heart disease between active and inactive men.—S. O. WAIFE

Further confirmatory evidence of the role of increased fat consumption in elevating the serum levels of cholesterol and beta-lipoprotein is presented in the important papers below. However, there are considerable data accumulating to suggest that the type of dietary fat is of importance in this relationship. There is not complete agreement as yet, but some workers have found a reduction of serum cholesterol following the feeding of unsaturated fatty acids.

Serum-cholesterol, Diet and Coronary Heart Disease. An Inter-racial Survey in the Cape Peninsula. B. Bronte-Stewart, A. Keys, and J. F. Brock. *Lancet* 2: 1103, 1955.

This report describes a study of the serum cholesterol and serum lipoprotein levels, diet, and personal habits of 383 men between the ages of 40 and 58 living in the Cape Peninsula. They were Bantu, Cape Coloured, and European, three racial groups which show striking differences in the incidence of coronary heart disease.

There were marked and highly significant differences in the mean total-cholesterol level and particularly in the cholesterol level in the β-lipoprotein fraction of the serum between the groups. The Bantu had the lowest, the Cape Coloured were intermediate, and the Europeans highest.

The levels were related to the fat consumption and rose within each group as income rose. "The results of this survey support the hypothesis that the dietary intake of fat influences the level of the serum cholesterol, particularly that of the β-lipoprotein, and in turn may be one of the major factors influencing the pathogenesis of coronary heart disease." This is interesting new evidence for this important hypothesis and should be read in the original by those working in the subject.—F. E. HYTTEN.

Effects of Feeding Different Fats on Serum Cholesterol Level. B. Bronte-Stewart, A. Antonis, L. Eales, and J. F. Brock. *Lancet* 1: 521, 1956.

A previous survey in the Cape Peninsula has revealed a widely different incidence of coronary heart disease between the different races, associated with parallel differences of serum cholesterol levels.

In this study the following eight males were examined: six (one Cape Coloured and five Bantu) with low serum cholesterol values and accustomed to a low fat diet, and two Europeans with coronary heart disease, high serum cholesterol levels, and accustomed to a diet rich in animal fat.

The two Europeans were maintained on a constant diet containing about 50 g/day of animal fat and the non-Europeans on a basically maize and bread diet free from cholesterol and containing very little fat. To the basic diets various fats and oils were added in amounts of from 100 to 200 g.

The results are presented in detail, but, briefly, animal fats and hydrogenated ground nut fat raised the serum cholesterol, whereas natural ground nut oil, sunflower seed oil, and marine oils depressed the serum cholesterol even in the presence of considerable quantities of animal fats. These changes were not affected by differences of protein, calorie, and vitamin intake.

It was demonstrated by feeding various fractions of the fats that the unsaturated fatty acid fraction caused the depression of serum cholesterol. The findings are discussed in relation to coronary heart disease.—F. E. HYTTEN.

Dietary Factors Affecting the Level of Plasma Cholesterol in Humans. The Role of Fat. J. M. R. Beveridge, W. F. Connell, and G. A. Mayer. *Canad. J. Biochem. & Physiol.* 34: 441, 1956.

Various workers have reported that vegetable fat in the diet does not have the propensity of raising or maintaining blood cholesterol levels possessed by animal fat. In this detailed report the investigators from Queen's University, Kingston, Ontario, found evidence that corn oil contains a factor (or factors) which leads to a depression of plasma cholesterol levels, while fats of animal origin (butter, lard, chicken fat, beef drippings) contain varying amounts of factors that increase cholesterol levels.

The studies were performed on large groups of students and faculty. After a period on a homogeneous formula diet (containing 58 per cent of the calories from corn oil) transfer to equicaloric diets containing animal fats led to plasma cholesterol increases. Furthermore, transfer from a mixed free-choice diet to one which was fat-free produced less of a fall in plasma cholesterol than a similar transfer to a diet containing corn oil.

These interesting observations lend further fuel to the animal vs. vegetable fat debate, and, because of the importance of the subject, deserve further study.—S. O. WAIFE.

The use of sitosterol to reduce serum cholesterol levels continues to engage the attention of researchers. Present evidence indicates that the desired cholesterol depression obtains when adequate doses (up to 30 g or more) of the beta form of this agent are given daily.

The Effect of Sitosterol Administration upon the Serum Cholesterol Level and Lipoprotein Pattern. C. Joyner, Jr., and P. T. Kuo. *Am. J. Med. Sc.* 230: 630, 1955.

Contradictory reports have appeared upon the effect of sitosterol upon blood cholesterol and lipids in human subjects. In the present study, the serum cholesterol level and lipoprotein pattern were measured to observe the effect of sitosterol on patients on an unrestricted diet and on a low fat diet using various doses and forms of the agent. It was found that beta-sitosterol produced a reduction in serum cholesterol concentration on unrestricted diets regardless of their initial blood cholesterol level. No untoward side-effects were encountered nor was there a significant weight change. The minimum effective dose was found to be above 10 g daily for a four-week course of treatment. Two patients maintained on a low fat diet evidenced a further lowering of serum cholesterol upon the addition of sitosterol. The electrophoretic lipoprotein patterns during the treatment program revealed a decrease in the large beta lipid zone including the tall beta peak and extending to the cathode side of the point of application. There was no significant alteration in the alpha lipid fraction or in the protein pattern. The authors state that a reduction of lipid in the beta-globulin fraction and of serum cholesterol may be effected with sitosterol without dietary fat restriction.—C. R. SHUMAN.

The Serum Cholesterol and Other Lipids after Administration of Sitosterol. J. M. Barber and A. P. Grant. *Brit. Heart J.* 17: 296, 1955.

A high level of dietary fat has been increasingly implicated in the pathogenesis of atherosclerosis. Severe restriction of dietary fat is, however, impractical, since the exclusion of fat makes for an unpalatable diet. Attempts are therefore being made to reduce the hypercholesteremia associated with high fat diets by the administration of certain plant sterols which have been shown to reduce experimental hypercholesteremia.

In this trial, the subjects were 26 patients with coronary artery disease and initial serum cholesterol values of at least 230 mg per 100 ml. For periods up to 21 weeks they received 9 g/day of almost 100 per cent pure beta-sitosterol (a plant sterol), incorporated in biscuits, and administered three times daily before meals. The biscuits proved acceptable, and there were no side-effects. The patients' diet was not restricted.

In 24 of the 26 patients, the minimum serum total cholesterol levels observed during the sitosterol regimen were lower than the initial level; in 17, the mean level was lowered. There was an increase in neutral fats

in 24 of the 26 patients, and the total lipids were significantly increased by sitosterol administration.

These results were not as striking as those reported by other workers. The reductions in serum cholesterol are "suggestive" rather than "significant." Dosage may have been a factor. Some investigators believe that doses under 10 g daily are without effect, and the authors of this paper have since noted a rapid and consistent fall in serum cholesterol when the dosage was increased to 18 g daily.

It is of interest that one patient who showed a reduction in serum cholesterol after sitosterol died, nonetheless, of a further myocardial infarction.—C.-J. HOWELL

Fat restriction to extremely low dietary levels has been recommended for treatment of patients with coronary artery disease for several years. While these measures are often impractical, further observations on clinical results are desirable.

A Nutritional Program for Prolongation of Life in Coronary Atherosclerosis. L. M. Morrison. *J. A. M. A.* 159: 1425, 1955.

One hundred proved cases of myocardial infarction were divided into two equal groups, one of which was placed on a diet restricted to 20 to 25 g fat daily. Other "customary medicaments" were continued. After 8 years, 76 per cent of the control group, ingesting a nonrestricted diet, were dead, compared to only 44 per cent of the low fat diet group.

The "estimated" dietary intake of the low fat group was 1600 cal, 225 g carbohydrate, 120 g protein, and 50–70 mg cholesterol a day. A supplementary multivitamin capsule was prescribed, together with a minimum of 2 oz of whole-wheat germ and 8 g of powdered brewer's yeast.

After three years, the treated males lost an average of about 20 pounds, and females about 17 pounds. No significant weight changes occurred after that in this group, nor in the control group at any time. The author concludes that the survival rate of patients with coronary atherosclerosis "was more than 100% greater" when on a specific diet than when on a "typical American diet."

While the survival rates *per se* are significant, much information is lacking which would determine whether or not the conclusion is significant. One would like to know, for example, the degree of dietary control of these patients; all that is mentioned is that co-operation appeared to be assured by the statements of the patients and their families. The age and sex distribution in the original groups, the method of follow-up, and the degree of disability are just a few unanswered questions. It is hoped that more data on this study will be supplied in order to evaluate properly the value of this regimen.—S. O. WAIFE

Lipoproteins and Diet in Coronary Heart Disease. T. P. Lyon, A. Yankley, J. W. Gofman, and B. Strisower. *California Med.* 84: 325, 1956.

This is a follow-up study for a five-year period of 351 patients with myocardial infarction and 119 patients with angina pectoris. In the report stress is placed on the "atherogenic index" devised by the authors, which is a mathematical expression involving the lipoproteins of the S_f 0–12, and 12–400 classes. In the experience of these workers a close correlation exists between certain values of this index and clinical coronary atherosclerosis.

A diet limited to 50 g fat and 200 mg cholesterol was recommended to the patients. After 5 years the 143 patients who stated they did follow this diet had lower lipoprotein levels and atherogenic index values than 74 patients who did not follow the diet. The myocardial infarction recurrence rate was about four times as high in the non-dieters as in the dieters.

The data were obtained from consecutive cases without knowledge of the lipoprotein level. There is no way of judging the extent of adherence to the diet nor of ruling out the possibility that other nondietary factors may have operated. Nevertheless, it is suggestive that this diet had beneficial effects and that the mechanisms probably involve the lipoproteins.—S. O. WAIFE

The Serum Lipid Pattern in Hyperthyroidism, Hypothyroidism and Coronary Atherosclerosis. R. J. Jones, L. Cohen, and H. Corbus. *Am. J. Med.* 19: 71, 1955.

Serum lipid fractionation and lipoproteins were studied in 25 hyperthyroid, 17 myxedema, and 17 coronary artery disease patients. The latter group was selected to match the myxedema patients from a large number of atherosclerotic individuals who had been followed previously. The mean total lipid, total cholesterol, and phospholipid levels were higher in the latter two groups than in the former. The beta-lipoprotein and faster rising lipoprotein classes were quite elevated, contrasted with insignificant changes in the alpha-lipoprotein. The increase in serum lipids in myxedema is qualitatively indistinguishable from that of idiopathic hypercholesterolemia. However, ultracentrifugation offered finer differentiation in that the beta-lipoprotein levels were higher in atherosclerosis for a given degree of hypercholesterolemia. This tends to support the concept that the primary disturbance in serum lipids in atherosclerosis is an elevation of the beta-lipoprotein level.—C. R. SHUMAN

NUTRITION AND PREGNANCY

There is a recognized need for increased protein, calorie, and accessory food factor intakes during pregnancy, in order to reduce the increased rate of toxemia and prematurity which attends undernutrition in these patients. However, the pre-pregnancy nutritional status of the patient is an important consideration in dietary planning. Since obesity is associated with a high incidence of toxemia, consideration should be given to the need for some degree of caloric restriction, with protein

intake adequate to avoid endogenous protein catabolism. However, since underweight does not always imply undernutrition, (according to evidence from body compartment studies), the desirability of increasing body weight during pregnancy to an extent above that necessary for the fetus is open to question, unless nutritional deficiencies are present as disclosed by careful dietary history and objective findings.

The Underweight Patient as an Increased Obstetric Hazard. W. T. Tompkins, D. G. Wiehl, and R. McN. Mitchell. *Am. J. Obst. & Gynec.* 69: 114, 1955.

Various catastrophes of pregnancy are found in a study of the Nutritional Research Clinic of the Pennsylvania Hospital to be the result of metabolic dysfunctions. The patient's weight at the time she becomes pregnant is very important. Markedly underweight patients have a greater than average chance of toxemia and a greatly increased probability of premature labor. Patients who were twenty per cent or more overweight or underweight have an increased probability of developing toxemia. These rates, however, are not statistically significant.

The incidence of prematurity is lower for overweight patients than for those of approximately normal weight, and decreases sharply for underweight patients. Weight changes during pregnancy are important. The increase of normal patients is 24 pounds. The risk of toxemia was greater among patients who were 20 per cent or more underweight.

The increased risk of developing toxic symptoms can be recognized by gaining above or below the "normal" gain during the second and third trimester. Excessive gain in the last month, however, is due in part to edema, which is a sign of physiologic dysfunction. Failure to attain the average gain during the second trimester should be considered as an early warning.

The frequency of premature labor was more than twice as great in patients showing less than the average gain, or a loss in weight in the first six months. In the case of the underweight patient, every effort must be made as early in pregnancy as possible to prevent the onset of spontaneous premature labor. This danger can be recognized on the described pattern.

The successful support of pregnancy will require an added intake of essential nutrients at an early stage. An average or greater gain in weight by underweight patients reduced the percentage of babies that weighed five pounds or less at birth. The relation of the weight of the mother at the beginning of the pregnancy and her prenatal gain of weight, with the weight and size of the baby has been studied. There is no indication that a relatively high rate of gain by underweight mothers does increase the size of the baby. The increase in her own tissue may afford greater protection to meet the stress of pregnancy. Failure to gain an average amount, especially in the first six months, increases the likelihood of premature labor, but greater gain has little or no effect on the size of the baby.—R. TAUBER

Incidence of Prematurity in Relation to Maternal Nutrition. P. C. Jeans, M. B. Smith, and G. Stearns. *J. Am. Dietet. A.* 31: 576, 1955.

The incidence of prematurity among infants rose sharply with decrease in the nutritional status of their mothers, according to the data on 404 births reported by these authors.

The women under study were generally of low income and few had had prenatal care or advice when they were admitted to the obstetrics ward of the University hospital about two weeks before delivery. Dietary habits of these women were reported in detail earlier (*J. Am. Dietet. A.* 23: 27, 1952). In review it was found that the mothers consistently consumed high intakes of white bread and potatoes. Only carrots, apples, and tomatoes were eaten with any frequency. Milk intake was low and caloric overindulgence was far more common than caloric inadequacy.

For purposes of comparing dietary intake with the incidence of prematurity, the maternal subjects were divided into five groups according to their daily protein intakes. These intakes were 85 g or more; 70–85 g; 60–70 g; 50–60 g, and less than 50 g. In these groups there were 37, 87, 103, 101, and 46 women, respectively. Numbers of prematures born to mothers of these groups were 2, 3, 4, 5, and 12 when prematures were defined as infants weighing less than 2500 g at birth.

The diets of the 12 women in the group consuming the least protein were uniformly very poor in calcium, protein, and riboflavin and were poor in ascorbic acid, vitamin A, thiamine, and iron. The caloric intakes of 11 of the 12 were below the recommended allowances. Two-thirds of the babies born to the women in this group were described as in good condition at birth; one with severe congenital anomalies was stillborn; another with an imperforate anus was strong enough to survive operation and lived. The smallest infant (750 g) died on the third day, and a fetus, not weighed, age estimated as seven months, died shortly after birth.

In summarizing the data, the authors point out that 4 per cent of the 227 mothers receiving 60 or more grams of protein daily gave birth to premature infants, whereas premature infants were born to 9.6 per cent of the 177 women receiving smaller amounts of protein. This difference was significant at the 2 per cent level, and when data on single births only were compared, the findings showed differences at the 1 per cent level.

These findings concur with those reported earlier by Burke, Harding, and Stuart (*J. Pediat.* 23: 506, 1943) who observed that infant weight and length at birth tended to parallel the mother's protein intake during the last two trimesters of pregnancy.

The authors state, in summary, that premature delivery among the better nourished women tended to be associated with frequency and total number of pregnancies and with multiple birth, whereas among the least well nourished, the incidence of prematurity showed less relationship to number or frequency of pregnancies.

The findings of the study speak vividly for the desirability of good nutrition during pregnancy to protect against small, weak infants. It does not claim that all birth abnormalities can be avoided through diet, nor does it implicate any one nutrient as being protective against prematurity.—J. M. SMITH

Diets of Pregnant Women. Influence of Socio-Economic Factors. G. H. Murphy and A. W. Wertz. *J. Am. Dietet. A.* 30: 34, 1954.

The nutrient intake of 65 pregnant women and the adequacy of their diets in relation to social and economic factors were studied. The women, ranging in age from 16 to 35 years, were patients of a physician in a New England college town. They received no diet instructions from the authors but were given those used routinely by the physician during prenatal care. Seven-day measured food intakes were recorded at three times during the study, i.e., during the third to fourth month of pregnancy, during the ninth month, and at three months post partum. These intakes were assayed by standard food tables for individual intake of several nutrients. Nutrients reported were calories, protein, calcium, phosphorus, iron, vitamin A, thiamine, riboflavin, niacin, and ascorbic acid.

Nutrient intakes were compared to the recommended allowances of the National Research Council (1948 revision) and grouped into those over 66 per cent of the recommendation, or under 50 per cent, and those which fell between these two points. It was considered that an intake greater than 66 per cent was reasonably adequate. On the basis of this comparison, it was found that "the intake of dietary calcium in late pregnancy was outstandingly poor," as 63 per cent of the women received less than 66 per cent of the recommended allowances of this nutrient. Forty per cent received less than two-thirds the allowance of iron, and thirty per cent were below this criterion in protein, thiamine, and ascorbic acid.

There was no difference in intakes as estimated during early and late pregnancy; but a significant decrease in calcium, phosphorus, riboflavin, and ascorbic acid occurred at 3 months post partum, which probably indicates that some attention had been given to improved nutrition during early pregnancy. The authors point out that the dietary intake post partum was more satisfactory than during pregnancy, since recommendations for the nonpregnant woman are much lower.

Information on the husband's occupation, income, the money spent for food, family size, and other pertinent factors obtained by interview were utilized in a study of the extent to which dietary adequacy was related to these factors.

This portion of the paper is difficult to follow, since tables are complicated by several factors and methods of statistical treatment are not clear. The authors conclude that for the group studied there was no trend as to the adequacy of the women's diets as related to the cost per week per food expenditure unit. Forty-three per cent of the 14 women who spent the most for food had diets that scored lowest in terms of adequacy. The percentages of low scores in the other two groups of women who spent less for food, were approximately the same.

As the size of the family increased, the money spent per food expenditure unit decreased. This did not affect the dietary quality of the mother's diet.

When the families were grouped according to the father's occupation into professional, student, trade and business, and unskilled labor groups, the authors found a definite relationship between the adequacy of the mother's diet and her social level: the wives in the professional and student groups had more adequate diets than those of the other two. This was not an income factor, since the student wife had the lowest income.—J. M. SMITH

The interpretation of blood levels of amino acids as representative of absorption rates may be open to question. It is reported below that low blood levels in a severely pre-eclamptic patient indicate impaired absorption. However, such levels may actually indicate (a) rapid urinary excretion or (b) a greater volume of dilution in the edematous patient.

Plasma Amino-Acid Levels Following Protein Ingestion by Pregnant and Non-pregnant Subjects. C. A. B. Clemetson and J. Churchman. *J. Obst. Gyn. Brit. Emp.* 62: 390, 1955.

The plasma level of amino acids is lower in pregnant than in nonpregnant women or in men. An interpretation of this has been that it is due to the active transfer to the fetus, but there has been no evidence of the behavior of the amino acid level after a protein meal.

In this enquiry, 100 g of a preparation of calcium caseinate ("Casilan") was given to 12 fasting subjects, and 8 blood samples were taken at intervals during the following 24 hours for amino acid analysis. There were 4 men, 4 nonpregnant women (between 7 and 10 days post partum), and 4 pregnant women of whom one had essential hypertension and another severe pre-eclampsia. The absorption curves are shown for each case. Except for the severe pre-eclamptic, who had a low curve of delayed absorption, and as far as can be determined with these small numbers, there was no systematic difference in the shape of the curve between the groups. The plasma amino acid level rises to a maximum at about three hours and then falls to reach the fasting level at about 12 hours.—F. E. HYTTEN

Tocopherol preparations used in human pregnancy to prevent habitual abortion have given unimpressive results. The following study reveals no relationship between plasma tocopherol concentrations and complications of pregnancy.

The Vanderbilt Cooperative Study of Maternal and Infant Nutrition. VII. Tocopherol in Relation to

Pregnancy. M. E. Ferguson, E. Bridgforth, M. L. Quaife, M. P. Martin, R. O. Cannon, W. J. McGanity, J. Newbill, and W. J. Darby. *J. Nutrition* 55: 305, 1955.

The role of tocopherol in human nutrition is undefined and studies of the influence of this vitamin on human pregnancy have been indecisive. Few estimates exist of the human dietary intake of vitamin E. However, several reports of the levels of total tocopherols in the blood during pregnancy agree that a rise occurs as gestation progresses.

The variations in plasma tocopherol levels during pregnancy are described based upon (a) 1575 single determinations of tocopherols and (b) a group of serial estimations on 39 women. The calculated dietary intake of total tocopherol for 195 pregnant women ranged from 2.9 to 33.3 mg, with a median approximating 10 mg. The average total tocopherol content of plasma increased from 0.89/100 ml for measurements during the first trimester of pregnancy to 1.40 mg/100 ml at 39 weeks or later. Increased concentration of tocopherol in plasma occurred with age and in diabetics. Plasma levels of tocopherol tended to be lower in the groups with the lower calculated intakes; the differences were noted at an intake level below 8 mg of total tocopherol daily.

An association exists between concentrations of tocopherols and carotene in the plasma. Significant deviation of maternal plasma tocopherol levels from the total average did not occur in any of the subgroups of subjects who experienced complications of pregnancy.

The authors conclude that no support has been obtained for the thesis that tocopherol deficiency accounts for the unexplained complications of pregnancy which are encountered in a "normal" obstetrical population as represented by the group observed in this study.—B. SURE

An increased utilization of riboflavin during pregnancy has been demonstrated, paralleling observations on ascorbic acid and other nutrient factors.

The Riboflavin-Excretion with Urine in Pregnancy. A. P. Jansen and B. C. P. Jansen. *Intern. Ztschr. f. Vitaminforsch.* 25: 193, 1954.

The riboflavin content of 167 samples of 24-hour urines of pregnant women (in the Netherlands) was determined by the lumiflavin method. Riboflavin intake over a 3-day period was estimated before urine sampling. The studies were done on women in the second to ninth month of pregnancy, who were divided into four groups on the basis of riboflavin intake: (1) up to 1.0 mg/day; (2) 1.0–1.5 mg/day; (3) 1.5–2.0 mg/day; (4) over 2.0 mg/day. (NRC recommended dietary allowances for riboflavin, 2.5 mg (pregnancy), were met in less than 5 per cent of the women.) For comparison, 24-hour urine samples (82) were collected from nonpregnant women and analyzed for riboflavin. (Of the nonpregnant group, only 24 per cent met the NRC allowance of 1.5 mg riboflavin/day.)

Although the riboflavin intake of pregnant women was higher than that of nonpregnant women, the former excreted less riboflavin than the latter.

Significance of the differences in riboflavin excretion by the two groups was calculated by means of Wilcoxon's test. At an intake of under 1.0 mg/day, the difference was not significant, but at an intake of 1.0–1.5 mg, it was highly significant, and very significant, too, at an intake of 1.5–2.0 mg. At intakes of over 2.0 mg, the differences were no longer significant, suggesting saturation of the organism with the vitamin, and indicating that the requirement for pregnancy is about 2.0 mg/day. (This figure, while lower than the NRC recommendation, was met by only 20–25 per cent of the pregnant women.)

Comparing the pregnant women as a whole with the nonpregnant women, the riboflavin excretion of the former was still significantly lower, despite a considerably higher intake.

These findings confirm that the riboflavin requirement is increased in pregnancy—certainly during the last six months, and probably, to a lesser extent, during the first trimester.—C.-J. HOWELL

IRON METABOLISM

The following studies on hemoglobin concentration and the results of iron administration in infants, children, and adults are of great interest in that they can be correlated to demonstrate trends in hemoglobin and erythrocyte formation with the passage of years from the neonatal period to maturity.

Studies of Iron Requirements in Infants and Children. I. Normal Values for Serum Iron, Copper and Free Erythrocyte Protoporphyrin. P. Sturgeon. *Pediatrics* 13: 107, 1954.

Three groups—normal adult males, normal infants from six to twelve days, and normal infants and children from two weeks through fifty-two months—have been studied by extremely well-controlled methods for the following: (1) serum iron level; (2) latent iron-binding capacity; (3) total iron-binding capacity (T.I.B.C.); (4) per cent saturation [(serum iron/T.I.B.C.) x 100]; (5) serum copper; (6) erythrocyte protoporphyrin; and (7) Hb per 100 ml, red cell count and the common indices.

The following conclusions were reached: On an average, the newborn infant serum, compared to that of the normal adult male, is hyperferremic (serum iron 193 μg/100 ml), hypocupremic (serum copper 51 μg/100 ml), and his free erythrocyte protoporphyrin is increased about two-fold (erythrocyte protoporphyrin 55 μg/100 ml). His total serum iron-binding capacity appears to be 50 per cent or more saturated.

At 12 hours of age, marked *hypo*ferremia (serum iron 46 μg/100 ml) is present and the total iron-binding

capacity as measured in this study is 100 per cent saturated. There is slight, if any, change in the serum copper or erythrocyte protoporphyrin during the first few hours or days of life.

From two weeks through one month of age, an essentially normal pattern is present except for the slightly elevated (two-fold) protoporphyrin.

By 4 to 10 months of age, on an average, the normal infant has the pattern characteristic of infancy, namely, hypoferremia (serum iron, 50 μg/100 ml), hypercupremia (serum copper, 146 μg/100 ml), reduced saturation of the total serum iron-binding capacity (12 per cent) and elevated free erythrocyte protoporphyrin (70 μg/100 ml).

Extreme ranges in values are found in different individuals at most ages.

The suggested explanation for these findings is that relative to the adult, on an average the normal infant is totally depleted of his iron reserve and that his state of "physiologic anemia" constitutes an additional, but lesser, iron deficit.

Unless due consideration has been given to the concentration of methemalbumin, hematin, and other intermediary products which make their appearance during the synthesis and degradation of hemoglobin, it appears that the high iron levels reported at birth may be somewhat erroneous. It has been observed (Tuttle) that methemalbumin may be abnormally elevated in as many as 50 per cent of normal infants during the immediate neonatal period, and it has been suggested that placental degradation of hemoglobin may play an important role.—J. N. ETTELDORF

Studies of Iron Requirements in Infants and Children II. The Influence on Normal Infants of Oral Iron in Therapeutic Doses. P. Sturgeon. *Pediatrics* 17: 341, 1956.

In a study undertaken to determine whether iron deficiency existed in infancy (see previous abstract), the author studied hemoglobin, RBC, MCV, MCHC, serum Cu and Fe, erythrocyte protoporphyrin, T.I.B.C. and the cytological and chemical values of the blood before and after the administration of 90 mg of elemental iron per day for a six-week period. Forty infants with an average age of 14 months, whose hemoglobin was 9 g per 100 ml or higher were selected.

Comparison of the above values before and after 6 weeks of therapy with standards for adults reveals a continuing iron-deficient state. However, a significant increase in hemoglobin concentration could be demonstrated from the administration of iron when a comparison was made with control groups of similar age selected at random from the normal population. Control groups were selected from comparable age groups at the beginning and from another group 6 weeks later. No significant changes in the remaining measured variables was observed. In six infants with a hemoglobin less than 9 g per 100 ml the increase in hemoglobin was greater than in the above.

The data suggest that 12 ± 1 g of Hb per 100 ml is the upper limit of normal beyond which iron therapy would be quite useless.

Iron deficiency of the normal infant population was confirmed by demonstrating hypoferremia, hypercupremia, reduced saturation of the total iron-binding capacity, and increased erythrocyte protoporphyrin.

These studies confirm the belief that "physiologic" anemia of *late* infancy can be significantly improved by increased iron nutrition. Although no cytologic changes were demonstrated, the author states that longer periods of administration, or iron started earlier in life, should and are being investigated.—J. N. ETTELDORF

The Total Body Hemoglobin in Children and its Relation to Caloric and Iron Requirements. W. W. Hawkins, V. G. Leonard, and E. Speck. *Metabolism* 5: 70, 1956.

In children 6 to 17 years of age, height, weight, and blood hemoglobin concentrations were determined to calculate surface area, blood volume, oxygen consumption, caloric expenditure, and the iron requirements for hemoglobin regeneration. Boys, at about age 14 years, manifest growth rates greater than those of girls with concomitant increases in total hemoglobin and blood volume. The blood hemoglobin concentration rises in boys at age 13 to 14, while, in girls, there is a slight decrease. The rate of decrease of the basal metabolic rate with age diminishes in boys; in girls the trend does not change. There is no significant difference between the sexes in the ratio of the oxygen in the systemic circulation to oxygen required for internal respiration.

The iron requirements in children increase with growth; however, in girls after 13 years catamenic losses represent the major requirement. The iron requirements increase from age 6 years to puberty. After puberty, the need for iron in boys to support the greater increase in hemoglobin outweighs that of girls, despite the menstrual losses in the latter.—C. R. SHUMAN

Hematopoiesis in Premature Infants with Special Consideration of the Effect of Iron and of Animal-Protein Factor. J. A. Wolff, and A. M. Goodfellow. *Pediatrics* 16: 753, 1955.

Hemograms (hemoglobin, RBC, WBC, differential, platelet, and reticulocytes) were followed in 81 premature infants. The infants were grouped according to weight: (1) those weighing less than 1200 g, and (2) those weighing between 1200–1500 g. Each of these groups was subdivided: (a) no treatment; (b) treated with iron, 16 mg of elemental iron daily; (c) given animal protein factor containing 5 mg of Achromycin, 1–5 mg vitamin B_{12} and 0.3 mg elemental iron. Iron therapy was given within the first 10 days of life.

No differences in the normal hemoglobin levels and erythrocyte counts were noted in the two weight groups, and therefore the degree of anemia which developed in

these premature infants could not be related to the birth weight. The lowest hemoglobin levels ranged between 7–8 g per 100 ml and occurred in the first 12 weeks of life.

One reticulocyte peak occurred immediately after birth, and a second peak at about 8 weeks of age corresponding to the greatest degree of anemia.

Iron therapy or animal protein factor containing vitamin B_{12} and Aureomycin had no statistically significant effect upon the early phase of anemia in these infants.

In the follow-up observations, it was clear that the iron-treated infants clearly benefited and had normal hemoglobin levels at one year of age; whereas the untreated infants remained anemic at this time. Blood transfusion is rarely necessary in the treatment of anemia of prematurity.

The initial platelet counts also were not influenced by the birth weight. The count at birth was 150,000/mm^3 and increased gradually between the sixth and twelfth week to 200,000–250,000. The leukocyte counts showed marked variation in all ages of both weight groups. The mean values are higher at birth and fall below 12,000/mm^3 by the fourth week. Segmented polymorphonuclear leukocytes predominate at birth and drop quite rapidly to 25 per cent at 3 months. The eosinophils are lower during the first 3 days than at any other time during the first 3 months. The lymphocytes at birth were approximately 33 per cent of the differential count and gradually increased to 60–70 per cent by the fifth week. The monocytes remained between 2–7 per cent, with no consistent change with increasing age.—J. N. ETTELDORF

The Concentration of Hemoglobin in the Blood of Young Adult Men and Women: The Effects of Administering Small Doses of Iron for Prolonged Periods. R. C. Garry, A. W. Sloan, J. B. de V. Weir, and M. Wishart. *Brit. J. Nutrition* 8: 253, 1954.

The accepted *normal* value for the concentration of hemoglobin in human blood has increased progressively with the passage of years. Whereas 13.8 g/100 ml blood was 100 per cent for Haldane's original twelve healthy men, something approaching 16 g/100 ml blood is now thought to be the desirable concentration in men. But the concentration in the blood of apparently healthy adult women, even when all factors other than sex are comparable, has obstinately lagged behind, so that something of the order of 14 g hemoglobin/100 ml blood is accepted as normal for them.

The literature dealing with the concentrations of hemoglobin in the blood of adult human beings is reviewed. Two series of observations were made on the concentration of hemoglobin in the blood of men and of women university students. In age and general environment the circumstances of these men and women were similar. Initially, although there was some overlap in the distribution, there was a statistically highly significant difference, in both series, between the means of the concentrations of hemoglobin in the bloods of the two sexes. In the first series the mean value for the 24 men was 15.92 ± 0.23 g Hb/100 ml blood; the corresponding figure for the 41 women was 14.20 ± 0.13. In the second series the mean value in 54 men was 16.21 ± 0.11, and that in 55 women was 14.59 ± 0.10.

In the first series the women received daily a supplement of 7 mg iron for a period of five months. The men received no additional iron. Although the mean concentration of hemoglobin in the blood of the women increased slightly, so also did that in the blood of the men. There was then no satisfactory evidence that giving a small dose of iron increased the concentration of hemoglobin in the blood of the women. In the second series the men and women were divided each into three groups. One group of the men and one group of the women received daily, in the form of a tablet, a supplement of 28 mg iron as ferrous sulphate. Copper and manganese, also as the sulphate, were present in the tablet to the extent of 0.52 and 0.47 mg, respectively. Two more groups, one of men and one of women, received a supplement of 14 mg iron with 0.26 mg copper and 0.24 mg manganese. The remaining two groups acted as controls, the subjects each receiving a placebo indistinguishable from the tablets containing iron. The administration of the tablets continued for one year.

At the end of the year the difference between the mean concentration of hemoglobin in the bloods of the men and of the women was still of the order of 1.5 g Hb/100 ml blood: administration of iron had signally failed to diminish the difference in concentration of hemoglobin in the blood of the two sexes. It is difficult, then, to attribute this difference to an inadequate intake of iron by the women. The groups of men and women that received iron showed, relative to the control groups, an increase in the concentration of hemoglobin in the blood. In both sexes, the lower the initial concentration of hemoglobin in the blood, the greater was the response to the administration of iron.—B. SURE

The absorption of iron occurs after reduction of this element to the ferrous state. This is facilitated by the reducing action of ascorbic acid provided by the diet. Apparently the intestinal mucosa serves to a considerable extent as a regulator of iron absorption, increasing the intake in hypochromic anemia and serving as a barrier when iron stores are normal. The amount of food iron excreted in the feces will depend largely upon the requirements for iron existing at the time of study. Apparently the adult male needs only 0.5 to 1.0 mg of iron daily, while the female may require nearly twice as much.

Iron from Gastrointestinal Sources Excreted in the Feces of Human Subjects. R. L. Ingalls and F. A. Johnston. *J. Nutrition* 53: 351, 1954.

Eight women 23 to 37 years of age served as experimental subjects. They varied in height from 145 to 168 cm and in weight from 48.6 to 64.4 kg. The hemoglobin values for the subjects were 12 g or more per 100

ml of blood except for two subjects, who participated only early in the study and for whom determinations were not made. Three experimental eight-day periods were each preceded by three days during which the subjects consumed a diet of self-selected foods but avoided foods high in iron. Each subject participated in the study following the end of menstrual periods; thus possible variations in absorption which might occur during the menstrual cycle were avoided. All 8 individuals did not continue as subjects throughout the study; 5 completed the diet for all three experimental periods, 2 completed the first period only and 1 completed the second and the third periods.

The young women were given diets, the mean content of which was 1.03, 2.15, and 3.22 mg of iron during three 8-day periods, respectively. The iron in the food and feces and the nitrogen in the food, feces and urine were determined.

The amount of iron in the feces from gastrointestinal sources was calculated by two methods. (1) A regression of the values for fecal-iron on food-iron was extended to zero. The calculated value for fecal-iron at zero intake was 0.17 mg. (2) The percentage absorption at which the gastrointestinal iron in the feces appeared to be the most constant for the three levels of intake was determined. By this calculation, the absorption for three intakes lay between 18 and 25 per cent and the value for gastrointestinal iron lay between 0.12 and 0.27 mg. By either method of calculation, the mean value for daily gastrointestinal iron in the feces approximated 0.2 mg.

To determine the relationship between the dry weight and the iron content of the feces, a correlation between dry fecal-weight and fecal-iron was calculated for each subject. The correlations were highly significant except for two subjects who had been given mineral oil and whose fecal excretions were delayed more than for the other subjects.—B. Sure

The use of AA′-Dipyridyl for Determining the Amount of Ferrous Iron Formed in the Digestive Tract of Women Before and After the Addition of Beef to the Diet. F. A. Johnston, R. I. Ingalls, and B. O. Muka. *J. Nutrition* 53: 83, 1954.

Two women consumed diets low in iron until the iron content of the feces reached a plateau. Then they were given 0.15 g of aa′-dipyridyl orally on each of two days after which they continued on the low iron diet for two more days. The subjects repeated the same procedure with beef substituted for a part of the sugar in the diet. On neither diet did the iron content of the feces rise upon the administration of aa′-dipyridyl. This indicated that aa′-dipyridyl does not combine with ferrous iron in the digestive tract to form an insoluble compound and cannot be used to measure the amount of ferrous iron formed, as claimed by previous investigators. One subject absorbed very little of the iron of beef and the other absorbed about one-third. —B. Sure

Serum Iron in the Diagnosis of Hepatobiliary Disease. L. Schamroth, W. Edelstein, W. M. Politzer, and N. Stevens. *Brit. Med. J.* 1: 960, 1956.

The differentiation between obstructive and non-obstructive jaundice may sometimes be difficult, even with available laboratory tests.

The serum iron was estimated 104 times in 63 patients with a variety of hepatobiliary diseases and was found to be significantly raised (above 220 mg per 100 ml) in almost all cases of viral hepatitis and to be below this level in nearly all cases of cirrhosis of the liver and extra-hepatic obstruction. Illustrative cases are described and the value of the test in diagnosis is discussed.—F. E. Hytten

Deposition of Iron in the Testes after Administration of an Iron-Dextran Complex. J. A. Nissim. *Lancet*, 1: 701, 1955.

The dextran-iron complex, Imferon, was injected intravenously into white adult mice in doses of 0.5 g of iron per kilogram of body weight weekly, or twice weekly until a total of up to 3 g per kg had been given.

Massive deposition of iron took place in the interstitial cells of the testes, and the seminiferous tubules were damaged, so that in a few weeks there was severe testicular atrophy. No corresponding deposition of iron was noted in any other endocrine organ and did not occur in ovaries.

It is not suggested that significant amounts of iron would accumulate in man after therapeutic doses of Imferon, more particularly in anemia when the iron would be rapidly mobilized for use in hemoglobin formation.—F. E. Hytten

THE ACTION OF VITAMIN K

Vitamin K was isolated from alfalfa in 1931 and has been found principally in leafy vegetables. Vitamin K_1 was isolated from putrified fish meal and was later synthesized from a potent quinone, menadione. Aqueous solutions for intravenous use have been prepared from water-soluble analogues and from colloidal preparations of the vitamin. The vitamin apparently functions as a coenzyme in the formation of prothrombin by the liver. The intravenous preparations will rapidly displace the antivitamin K preparations which are used to inhibit prothrombin production in the course of anticoagulant therapy. Slower return of prothrombin time to normal occurs with oral therapy.

Effect of Intravenous Vitamin K on the Action of Phenindione. P. Dawson. *Brit. M. J.* 2: 1427, 1955.

The effects of intravenous vitamin K on prothrombin activity were tested in 24 healthy volunteers during the administration of phenindione. Doses of 10 to 20 mg of vitamin K returned the prothrombin activity to 100 per cent within 24 hours in subjects who had taken, and were continuing to take phenindione (dose

not stated); 5 mg raised the prothrombin activity to between 50 and 100 per cent.

In more than half the experiments an appreciable rise occurred within three hours, but the speed of the response was not related to the size of the vitamin K dose.

"In the treatment of haemorrhage due to phenindione an intravenous dose of 10–20 mg. is likely to be effective." No side reactions were seen with the doses of vitamin K used.—F. E. HYTTEN

Antagonistic Effect of Oral Vitamin K_1 on the Action of Ethyl Biscoumacetate and Phenylindanedione. J. N. M. Chalmers, M. F. Dixon, and W. Polack. *Brit. M. J.* 2: 957, 1954.

Vitamin K_1 in the form of a fine emulsion was given by mouth in doses of 100 mg to normal healthy subjects whose "prothrombin activity" had been reduced by ethyl biscoumacetate (Tromexan) and phenylindanedione. In a series of experiments the "prothrombin activity" was reduced to approximately 10 per cent of normal and a single dose of 100 mg of vitamin K_1 was given while therapeutic doses of the anticoagulant were continued. Normal prothrombin activity was restored in from 24 to 30 hours.

If the anticoagulant drugs were stopped when the vitamin K_1 was given, "prothrombin activity" returned to normal in an average of about 10 hours. Doses of 30 mg of vitamin K_1, given during anticoagulant therapy, raised the prothrombin activity by about 20 per cent in from 6 to 12 hours, and this was considered to be a useful controlling dose for ordinary purposes.

The authors consider that the practical importance of the vitamin K_1 emulsion is that it "can rapidly modify an unduly low or potentially dangerous drug-induced hypoprothrombinaemia or correct it in the emergency of haemorrhage."—F. E. HYTTEN

Vitamin K_1 in Anticoagulant Therapy. M. Toohey. *Brit. M. J.* 1: 1020, 1954.

The use of vitamin K_1 in antagonizing the action of anticoagulant drugs in 70 patients is described. It is stressed that vitamin K_1 is a powerful drug in this respect and its use must be carefully controlled. A failure of response to it is an ominous prognostic sign, and the only two patients in this series in whom it was ineffective were extremely ill and died of myocardial infarction.

There is a useful guide to the use and dosage of vitamin K_1 in varying circumstances which depend on the prothrombin level of the blood, the type of anticoagulant in use, and certain clinical features.—F. E. HYTTEN

Bile salts are necessary for the absorption of vitamin K from the small intestine. As it is a fat-soluble vitamin, bile is necessary for the emulsification and passage of vitamin K into the lacteals from whence it passes into the thoracic duct.

Vitamin K Requirements of Adult Dogs and the Influence of Bile on Its Absorption From the Intestine. A. J. Quick, C. V. Hussey, and G. E. Collentine, Jr. *Am. J. Physiol.* 176: 239, 1954.

Dogs were prepared with cholescystonephrostomies. This excludes bile from the intestines: deficiencies of vitamins A and K result. Using this preparation it was determined that 0.5 μg of natural vitamin K_1 per kilogram of body weight is required to maintain a normal prothrombin level. Five ml per kilogram of body weight of bile provide enough vitamin K for absorption to restore prothrombin levels to control values. Oral vitamin K_1 without bile will restore prothrombin levels if large enough doses are used.—M. J. OPPENHEIMER

THE PANCREAS AND LIPIDS

The secretions of the pancreas influence lipid metabolism both in the absorption and utilization of fats. Pancreatic lipase is important in promoting the hydrolysis of neutral fats to fatty acids and glycerol. To some extent, however, this function can be supported by lipases derived from the succus entericus, so that the digestion and absorption of fats can proceed. The administration of pancreatic extracts improves fat absorption in patients with pancreatic disease, indicating the probable reduced availability or efficacy of nonpancreatic lipases in maintaining fat digestion.

Non-pancreatic Lipase in Children with Pancreatic Fibrosis. C. A. C. Ross and H. G. Sammons. *Arch. Dis. Child* 30: 428, 1955.

The fact that children with pancreatic fibrosis and no pancreatic lipase nevertheless absorb a considerable proportion of dietary fat suggests a source of non-pancreatic lipase.

Milk and fruits were examined as possible dietary sources and the feces and gastric juice for intestinal sources. Cultures of intestinal bacteria were also studied for their ability to produce an exo-lipase.

Fresh unpasteurized milk was the only food examined which contained lipase; human milk was much richer than cow's milk. Lipase was present in feces but not in gastric juice and was not made by bacteria. It therefore appeared to be produced by the succus entericus.—F. E. HYTTEN

The Effect of Pancreatin Therapy on Fat Absorption and Nitrogen Retention in Children with Fibrocystic Disease of the Pancreas. R. Harris, A. P. Norman, and W. W. Payne. *Arch. Dis. Child.* 30: 424, 1955.

Fat and nitrogen balances were studied in twelve children, between $4\frac{1}{2}$ and 10 years old, with fibrocystic disease of the pancreas, with and without pancreatin added to the diet. Both fat and nitrogen absorption was markedly improved by 5 g of pancreatin added to each meal. The addition of 15 g did not appreciably improve the results. There was a general clinical improvement.—F. E. HYTTEN

The utilization of fats is influenced by the pancreas indirectly under physiologic conditions by promoting the digestion of protein and absorption of antilipotropic factors necessary to prevent fatty infiltration of the liver. Evidence to support the role of the pancreas as a source of a factor (lipocaic) influencing blood and hepatic lipids directly is presented below.

Relation of the Pancreas to the Regulation of the Blood Lipids. L. R. Dragstedt, J. S. Clarke, G. R. Hlavacek, and P. V. Harper, Jr. *Am. J. Physiol.* 179: 439, 1954.

This study indicates that the pancreas has an important role in the control of blood lipid levels. In normal dogs, the values for total serum lipids are in the range of 500–900 mg per 100 ml. When nutrition is good, there is a tendency for blood lipids to be higher than when a poor nutritional state is present, although fasting does not produce any definite change. Depancreatized dogs were treated with insulin. In these animals serum lipids are low. However, more profound effects (lowering) are produced by ligation of the pancreatic ducts, which is followed by degeneration of pancreatic parenchyma. Lipocaic-deficiency symptoms (fatty liver, decreased hepatic function, decline in insulin requirement, and decreased glucose excretion) most often occurred at the time the blood fats were decreasing. A small remanant of pancreas attached to a duct was enough to produce diabetes, with a high dose of insulin needed in order to prevent glycosuria and intense hyperlipemia. It was unnecessary to give these animals pancreatic substance. However, if the pancreatic juice from this small remnant is carried to the exterior, the insulin requirement falls to a value like that in completely depancreatized dogs. There is now no hyperlipemia. An external pancreatic fistula does not produce fatty infiltration of the liver and a low blood fat level, if glycosuria is prevented by the activity of some normal pancreatic tissue. Fibrosis of the pancreas results when the ducts are ligated. This operation seems to produce an endocrine disturbance in the pancreas. This is shown by the fact that diabetes resulted when the ducts were tied from the remnant of a pancreas which had been able to preside over a normal carbohydrate metabolism. When fresh beef pancreas was given by mouth to depancreatized dogs, to those with ligated pancreatic ducts, or to animals with ligated ducts and partial pancreatectomy, a hyperlipemia resulted. On the other hand, similar feeding has no effect on normal dogs. The hyperlipemia of hypothyroidism and partial pancreatectomy were shown to be additive. The authors suggest that the internal secretion, lipocaic, from the pancreas regulates the fat levels of the blood. Lipocaic potentiates dietary choline and other lipotropic substances. A deficient endocrine pancreatic function (pancreatectomy or impaired activity) plus pancreatic juice in the intestinal lumen results in hyperlipemia and high insulin requirement. Conversely, if pancreatic juice does not enter the intestinal lumen when endocrine pancreatic function is depressed, a hypolipemia and decreased insulin requirement are the result. There is also decreased insulin tolerance and accumulation of liver fat.—M. J. OPPENHEIMER

Contradictory results are reported in the two following papers concerning the role of the pancreas in the absorption of cholesterol. If cholesterol absorption is effected by esterification with fatty acids made available through digestion of fats, one would have to assume that the pancreatic secretions are needed to promote cholesterol absorption.

Role of Pancreatic Juice in Cholesterol Absorption. H. H. Hernandez, I. L. Chaikoff, and J. Y. Kiyasu. *Am. J. Physiol.* 181: 523, 1955.

In this study a preparation is devised in which both pancreatic juice and bile are prevented from entering the intestinal tract. Cholesterol containing C^{14} was fed and the C^{14} was detected in thoracic duct lymph. Rats so prepared do not absorb cholesterol. If pancreatic juice is administered, absorption still does not take place. Bile, however, if given alone continuously, does restore absorption at reduced rates. Bile and pancreatic juice by continuous drip restore cholesterol absorption. Homogenates were prepared from duodenums of rats from which pancreatic juice had been excluded. These homogenates are almost completely unable to esterify cholesterol after 24 hours. This corresponds with the time at which rats are unable to absorb cholesterol.—M. J. OPPENHEIMER

Observations Concerning the Production and Excretion of Cholesterol in Mammals. XV. Role of the Pancreas in Intestinal Absorption of Cholesterol. S. O. Byers, and M. Friedman. *Am. J. Physiol.* 182: 69, 1955.

Cholesterol and lipid continued to be absorbed after pancreatic flow was excluded from the intestine. In fact, when pancreatectomy was subsequently performed on these same animals, it was observed that much cholesterol and lipid continued to be absorbed. It is apparent that large changes in the amount of pancreatic juice entering the intestine do not produce large alterations in cholesterol absorption.—M. J. OPPENHEIMER

Hypophysectomy has been shown to impair the function of various organs such as the kidney. In the following study a similar effect is observed in relation to the pancreas.

Effects of Hypophysectomy and of Undernutrition on Amylolytic Activity of the Pancreas of the Rat. J. L. Barrett, M. T. Nishikawara, and R. E. Haist. *Am. J. Physiol.* 182: 35, 1955.

Hypophysectomy reduces the size of the exocrine pancreas as well as that of the endocrine glands. When

the pituitary gland is removed there is a statistically significant decrease in the concentration of amylase activity of the pancreas. The total amylase is also less in hypophysectomized animals than in controls. Young rats were fasted 2–3 days. Although the pancreases of these animals weighed less than those of fed normal animals, there was no reduction in amylase activity if the basis of comparison was unit weight of gland. Total amylase was, however, reduced in fasted animals. Another group of rats of greater weight were fasted 5–6 days. In this case amylase activity was reduced no matter what the basis for comparison. If just enough food was allowed to maintain body weight, amylase activity was unchanged on the basis of unit weight of pancreas or body weight when compared to *ad libitum* fed controls. Nevertheless, total pancreatic amylase activity was reduced in these animals on restricted diet. As a result of these studies it is concluded that undernutrition does not explain the effect of hypophysectomy on the pancreas. There is a real action of the pituitary on pancreas exocrine function as measured by amylase activity.—M. J. OPPENHEIMER

PERNICIOUS ANEMIA AND VITAMIN B_{12}

Pernicious anemia is currently viewed as a deficiency disease involving vitamin B_{12}, the deficiency resulting from lack of absorption of the vitamin. Rather than a deficiency state, this disorder may actually represent an example of enzyme failure. The enzyme involved is a mucoprotein known as intrinsic factor, recently chemically identified, which is necessary for the absorption of vitamin B_{12} from the intestinal tract. The urinary excretion of radioactive vitamin B_{12} following an oral dose of this substance reflects intrinsic factor activity and provides a simple means for diagnosis of pernicious anemia. One exception may be sprue, in which impaired intestinal absorption is present and will lower urinary excretion of vitamin B_{12} despite adequate intrinsic factor.

The Urinary Excretion Test in the Diagnosis of Addisonian Pernicious Anemia. S. F. Rabiner, H. C. Lichtman, J. Messite, J. Watson, V. Ginsberg, L. Ellenbogen, and W. L. Williams. *Ann. Int. Med.* 44: 437, 1956.

Further confirmation of the value of the urinary excretion of orally administered radioactive vitamin B_{12} as described by Schilling, in the diagnosis of pernicious anemia is afforded by this article. The authors suggest one modification of the test as originally described, namely, the collection of two 24-hour urine specimens following the test dose. It was found that one "normal" subject excreted a suboptimal amount of the vitamin B_{12} Co^{60} on the first day, but the amount excreted on the second day placed him in the normal range, which is more than 5 per cent of the injected radioactivity. All of the patients with pernicious anemia excreted less than 3 per cent of the radioactivity administered to them.

Intrinsic factor of "known potency" was fed along with the vitamin B_{12} Co^{60} to 33 of the patients with pernicious anemia. This resulted in an increase in the urinary radioactivity in every patient, although in seven it failed to raise the value above the 5 per cent level. Thus one must assume that individual patients will require varying amounts of intrinsic factor, which of course reflects the well appreciated fact that patients with pernicious anemia have varying quantities of intrinsic factor in their gastric secretions. These observations then require that every patient to be treated with oral preparations for pernicious anemia should be assayed for intrinsic factor response before treatment is begun. That a uniform dosage level of intrinsic factor in a proprietary capsule will suffice for every pernicious anemia patient is a dangerous fallacy. Since competent radioisotope laboratories are not available to the large majority of practicing physicians, it appears that parenteral vitamin B_{12} is still the treatment of choice for pernicious anemia.

The urinary load test as described by the authors is valuable in the diagnosis of pernicious anemia in a patient who has received prior treatment, the details of which are unavailable to the physician, or in the patient with neurologic disease accompanied by minimal anemia.—J. F. MUELLER

Increased fecal excretion of radioactive vitamin B_{12} is a more tedious method of testing for intrinsic factor activity than the urinary test proposed by Schilling. In pernicious anemia, a greater proportion of the test substance undergoes fecal excretion due to lack of absorption.

Further Observations on the Oral Administration of Co^{60} Vitamin B_{12} to Normal Persons, Patients with Pernicious Anemia, and Subjects with Various Medical Disorders. L. M. Meyer, M. Jiminez-Casedo, and S. N. Arkun. *Acta med. Scandinav.* 153: 153, 1955.

Six normal subjects received 0.1 μg of radioactive (Co^{60}) vitamin B_{12} by mouth. The fecal radioactivity ranged between 23 and 49.9 per cent of the administered dose. Of a group of 39 patients with various medical disorders, fecal radioactivity was less than 50 per cent of the administered dose (except in one case each of cirrhosis, thrombocytopenic purpura, and postcolectomy). No relation was apparent between fecal excretion and the presence of free gastric hydrochloric acid.

The fecal excretion of radioactivity in 11 patients with pernicious anemia ranged from 57.5 to 98.1 per cent. Folic acid or pooled normal gastric juice reduced this excessive excretion in most cases. Some differences exist between these results and those reported from other laboratories, and the possible causes are discussed.—S. O. WAIFE

The following observations are of considerable significance in view of the current popularity of oral therapy for pernicious anemia. The question of the activity of intrinsic factor used in oral preparations has been raised. The continuation of activity of these preparations during long-term treatment is as yet undetermined.

Oral Treatment of Pernicious Anaemia with a Combined Vitamin B_{12} and Intrinsic Factor Preparation. E. K. Blackburn, H. Cohen, and G. M. Wilson. *Brit. M. J.* 2: 461, Aug. 20, 1955.

An important result of this trial was the discovery that the disadvantages of oral maintenance with vitamin B_{12} did not become apparent till the end of a six-month period. For the first six months the treatment seemed quite adequate, and the fact that ill effects were discernible later on indicates how cautious must be the acceptance of short-term trials of oral vitamin B_{12}.

An oral preparation containing 6 μg of crystalline vitamin B_{12} and 30 mg of "intrinsic factor" per tablet was administered to a group of patients with pernicious anemia. Five patients, previously untreated, were started on the tablet. In four of the five the response to 5 tablets a day was satisfactory. In the fifth, however, there was no response at all, and parenteral therapy had to be instituted; on this the patient did well. Twelve patients who had been successfully maintained for a year or more on parenteral vitamin B_{12} and were then switched to the oral preparation (1 tablet/day) were compared, one year later, with 12 comparable controls who had continued to receive parenteral vitamin B_{12}. No significant changes occurred in the mean hemoglobin values and red cell count during the first six months of oral therapy, but by the end of a year these values had fallen significantly in the group taking the oral preparation. Curiously, too, another patient, not included in this series, developed the clinical features of subacute combined degeneration of the cord while she was on oral maintenance therapy, despite the fact that her mean blood values were no lower than those obtaining while she was receiving the vitamin by the parenteral route. Increasing the oral dosage from 1 to 5 tablets per day led to temporary symptomatic improvement, but did not maintain it, and only when parenteral therapy was reinstituted were her symptoms eliminated.

The results of this trial suggest that the parenteral route is still preferable to the oral route of administration of vitamin B_{12}. The potency of crystalline vitamin B_{12} is known and its action is predictable, while the composition and effects of "intrinsic factor" preparations are variable. Until we completely understand the mode of action of intrinsic factor we cannot predict with any confidence the results of oral treatment with preparations containing it. The ultimate failure of oral maintenance after an apparent initial success in this whole group, the lack of response observed in one patient, and the development of rather serious symptoms in another are so many indications of the presence of therapeutic "unknowns." These authors are therefore convinced that any patient who requires vitamin B_{12} should receive it parenterally.—C.-J. Howell

A rise in vitamin B_{12} levels in patients with megaloblastic anemia receiving antibiotics, including penicillin, reported below, raises the question of increasing intrinsic factor activity under these conditions. Competition for vitamin B_{12} by intestinal micro-organisms has been repeatedly suggested as a cause for anemia in these cases. However, other possible effects need to be explored.

Penicillin in Megaloblastic Anaemias of Africans. Effect on Serum Vitamin B_{12} Levels and Absorption of Radioactive Vitamin B_{12}. H. Foy, A. Kondi, and P. E. C. Manson-Bahr. *Lancet* 2: 693, 1955.

Two types of nonpernicious megaloblastic anemia in Africans can be distinguished according to their response to treatment: one type responds completely to penicillin (200,000 units daily by mouth or 400,000 intramuscularly) or to 80 μg of vitamin B_{12} by mouth. In this type the serum vitamin B_{12} is low before treatment but rises with treatment; there is free gastric acid and the excretion of radioactive vitamin B_{12} after treatment is from 35 to 55 per cent of the administered dose. The most probable explanation of the response to penicillin in this type of anemia is that the antibiotic destroys micro-organisms in the gut which compete with the body for vitamin B_{12}.

The other type of megaloblastic anemia responds neither to penicillin nor to vitamin B_{12}, but does respond to folic acid by mouth. In this type, the serum vitamin B_{12} ranges from normal to very high before treatment.

The incidence of these anemias varies with the season, probably because of variations in diet, which may favor (or otherwise) the growth in the intestine of bacterial competitors for hemopoietic substances.—F. E. Hytten

Pernicious Anaemia in the South African Bantu. J. D. Woods and J. J. H. Rymer. *Lancet* 2: 1274, 1955.

Pernicious anemia is reported to be exceedingly rare among the Bantu. A case is described of a 50-year old pure-bred Bantu woman with a characteristic pernicious anemia and subacute combined degeneration of the spinal cord. The latter deteriorated during treatment with folic acid, but improved greatly with vitamin B_{12}.—F. E. Hytten

The influence of vitamin B_{12} upon the metabolism and utilization of methyl groups and the role of various methyl donors, particularly glycine, are nicely demonstrated in the following paper.

Vitamin B_{12} and Choline Synthesis from Glycine in vivo. B. C. Johnson, J. Firth, and S. P. Mistry. *Arch. Biochem. & Biophys.* 54: 467, 1955.

Previous experiments have shown that baby pigs on casein diets containing adequate vitamin B_{12} and approximately 0.8 per cent methionine, 2 per cent serine, and 0.6 per cent glycine required choline to prevent fatty livers and kidney damage. It has also been reported that the baby pig does not require choline if

given diets containing 1.6 per cent methionine. The present study indicates that a soy protein diet containing a total of 0.8 per cent methionine, 2 per cent serine, and 2.2 per cent glycine will completely protect baby pigs from choline deficiency in the presence of vitamin B_{12}. The latter diet supplies essentially the same amounts of serine, threonine, and histidine, but considerably more glycine than did their previous casein diet. In the absence of vitamin B_{12}, or in the presence of vitamin B_{12} but with the glycine level down to 1.6 per cent, choline deficiency results. Vitamin B_{12} is required for synthesis of methyl groups from glycine, and glycine, if present in sufficient amount, will serve as a satisfactory source for choline synthesis in the baby pig, but only in the presence of vitamin B_{12}.

Dimethylaminoethanol appears to have a sparing action on methyl synthesis, whereas aminoethanol greatly increases the methyl group demand, causing choline deficiency even in the presence of vitamin B_{12} and glycine at 2.2 per cent dietary level.—M. K. HORWITT

ITEMS OF GENERAL INTEREST

Red Blood Cell Niacin and Plasma Riboflavin Levels in a Group of Normal Children. M. N. Bartlett. *J. Nutrition* 57: 157, 1955.

Blood levels of some of the vitamins have been determined as part of a long-range study of normal children. Normal levels of red blood cell niacin, in a group of 86 children studied over a period of about 5 years, are presented. The results of these determinations are correlated with niacin intake and hematologic studies. No positive correlation was found. Normal levels of plasma riboflavin, in a group of 92 children studied over a period of approximately 5 years, are also presented. The results of these studies are correlated with riboflavin intake. No positive correlations were found. The results of both series of determinations are discussed in relation to theoretical considerations and in the light of other experimental findings.—B. SURE

Effects of Vitamin Supplementation of the Diet on Reaction to Short-Term Cold Stress in Normal Young Male Adults. E. P. Ralli, W. J. Kuhl, Jr., H. Gershberg, E. M. Beck, E. R. Street, and B. Laken. *Metabolism* 5: 170, 1956.

Immersion in water of 9.3° C for 8 minutes was used as the stressful event in normal male students in order to observe possible differences in responses before and after administration of pantothenate, vitamin B_{12}, B complex plus ascorbic acid, or placebo. The evaluation of the stress reaction was measured in terms of temperature, heart rate, blood pressure, differential leukocyte counts, serum sodium, potassium, protein, water, blood sugar, lactic acid, ascorbic acid, and cholesterol. Urinary excretions of uric acid, creatinine, ascorbic acid, and electrolytes were determined. The results disclosed that vitamin B_{12} therapy reduced somewhat the fall in temperature observed in all groups. No effect of therapy was noted upon blood pressure or pulse rate. Wide variations were noted in the leukocyte studies; the per cent fall of eosinophils was decreased in the group receiving pantothenate. Therapy had no effect upon serum water or lactic acid levels. The blood level of ascorbic acid was higher in those receiving ascorbic acid and calcium pantothenate. Vitamin B_{12} decreased the blood level of ascorbic acid. There was no effect upon sodium or potassium levels, although serum chloride levels were lower in the pantothenate and placebo groups after stress. It was noted that supplementation of the normal diet did not influence the capacity to recover from stress to any significant extent. High blood levels of ascorbic acid did not affect the capacity of these subjects to withstand cold stress.—C. R. SHUMAN

Measurement and Interpretation of Subcutaneous Fat, with Norms for Children and Young Adult Males. W. H. Hammond. *Brit. J. Prev. & Soc. Med.* 9: 201, 1955.

By the presentation of a great many data collected from about 4500 subjects, the use of skinfold calipers as a measure of subcutaneous fat is evaluated.

The M.R.C. dial-type caliper, with a plate size of 6 x 15 mm and a pressure of 10 g/mm² was used, and applied to 16 different sites. The skinfold measurement was compared to actual fat thickness as measured by x-rays.

The results are shown in considerable statistical detail with examples showing changes in skinfold measurements at different ages in boys up to the age of 18 and girls up to 15 in different nutritional grades and at different social levels. It is not possible to abstract the main bulk of this adequately and it should be studied in the original by workers in this field.

The broad conclusions are that any of the measurements made were highly representative of the total body fat and the technique is therefore a valuable anthropometric tool. When measurements at several sites are used in combination, the best measure of total fat was the biceps or triceps region, plus those at the subscapular and abdominal sites.—F. E. HYTTEN

The Serum Protein Pattern of Africans in Uganda. M. D. Thompson. *Tr. Roy. Soc. Trop. Med. & Hyg.* 50: 77, 1956.

It is well known that in tropical and subtropical countries the pattern of plasma proteins tends to be different from that found in Europe and North America. Although the total protein concentration may be normal or somewhat high, the albumin is low and the globulin, particularly the gamma globulin, is raised. Dr. Margaret Thompson has investigated this problem in infants in Uganda, on the hypothesis that the changes in pattern may be related to malaria. From birth up to the age of 6 or 9 months the absolute and relative

concentrations of the various protein fractions were the same in the African baby as in Europe or America. Thereafter the patterns diverge. In the European baby the albumin gradually rises after the first month, to reach the adult level at about 1 year. In the African it reaches a maximum at 6–9 months, and then falls again. At the same time the gamma globulin rises. Thus what one might call the "tropical pattern" is already established at 12–18 months. These changes in plasma proteins seem to be strikingly correlated with the incidence of malaria. The proportion of infants with a positive blood film rose from 30 per cent at 3 months to 80 per cent at 9–12 months.

The author concludes that there is no evidence of a genetic basis for the difference in protein pattern. The results fit the hypothesis of malaria as the cause, although a low protein diet may also play a part.

(It must be remembered that the same phenomenon has been observed, at least in adults, in countries where malaria is much less common—for instance in Jamaica and in Central America. A minor criticism of this paper is that more use might have been made of statistical methods. The conventional mean and standard deviation do not utilize all the available information. The numbers are rather small, the scatter wide. It would be interesting, for instance, to know whether in individuals as well as in groups there was a correlation between a positive malaria film and a high gamma globulin.)—J. C. WATERLOW

The Serum Protein Pattern of Africans in Uganda: Relation to Diet and Malaria. E. G. Holmes, M. W. Stanier, and M. D. Thompson. *Tr. Roy. Soc. Trop. Med. & Hyg.* 49: 376, 1955.

Serum protein determinations were made in adults and children in three parts of Uganda, which differed both in the nature of the staple diet and in the malaria incidence. In all three districts the pattern of the serum proteins was different from the normal European pattern, the total protein being somewhat higher, the albumin lower, and the gamma globulin considerably raised. There were also differences between the three African groups; no evidence was found that these differences were related to the protein content of the diet. On the other hand, in the children there was a correlation between abnormalities of albumin and globulin and the incidence of malarial parasitemia. In the adults this relationship was less clearcut, perhaps because the examinations were done in the dry season, when the malarial transmission rate is low.—J. C. WATERLOW

Thyroid Activity During Operation. I. S. Goldenberg, L. Lutwak, P. J. Rosenbaum, and M. A. Hayes. *Surg., Gynec. & Obst.* 102: 129, 1956.

For several years, Goldenberg and his associates have been attempting to assess the role of the thyroid in the metabolic reaction to operation. In the present study, attention is focused on the period of operation itself and the few hours immediately thereafter. As an index of thyroid activity, the authors have chosen serial measurements of serum protein-bound iodine (PBI^{131}). Their data are expressed in terms of a "conversion ratio." This is defined as follows:

$$\text{conversion ratio} = \frac{\text{net counts of } PBI^{131} \text{ in 2 ml serum}}{\text{net counts in 2 ml serum}} \times 100.$$

A patient was arbitrarily considered to have increased thyroid hormone levels if the conversion ratio increased more than 10 units over the earliest preoperative determination.

Sixteen patients of varying ages undergoing a variety of operative procedures were studied. Each subject was given a tracer amount of I^{131} two to three days prior to operation. The 24-hour uptake of I^{131} by the thyroid gland was measured. On the day of operation, blood samples for PBI^{131} determinations and measurements of radioactivity in the total serum were drawn prior to administration of the preanesthetic medication, after premedication, during induction of anesthesia, during the operative procedure and for several hours thereafter until the patient was fully awake. In most instances, the anesthesia was general and given by the endotracheal route.

There were seven patients who were undergoing their primary operative procedure at time of this study. Their ages ranged from 35 to 72 years. Preoperatively, their thyroid iodine uptakes had ranged between 30 and 49 per cent, while their conversion ratios varied from a low of 3 to a high of 49. In each of these individuals, there was an abrupt increase in the conversion ratio within the first hour following the start of induction; in about half the patients the conversion ratio was still elevated at the final observation five to ten hours later.

There were nine patients who were suffering from a chronic illness or a recent acute stress at the time of this study. Their ages were 46 to 78 years. The 24-hour I^{131} uptake preoperatively by the thyroid of these patients ranged from 26 to 69 per cent. This latter high figure was in a 46-year-old woman who had bleeding hemorrhoids necessitating transfusions preoperatively. Her preoperative conversion ratio (74) was also the highest in the series; in the postoperative period it was 81. The preoperative conversion ratios of the other patients in this group ranged from 4 to 64; in no instance was there an increase of more than 10 units during operation or in the immediate postoperative period.

One patient was studied through two major operative procedures. His problem was multiple colonic polypi. Following his first operation, an ileosigmoidostomy, there was an increase in the PBI conversion ratio; no increase followed the second operation 48 days later.

During the period of study, the hematocrits did not vary more than 3 per cent in any patient and in only one patient did the plasma protein levels change as much as 1 gram per cent.

The authors interpret these data as indicating an abrupt increase in thyroid activity in the operative

and very early postoperative period in previously unstressed patients.—S. M. LEVENSON

The Metabolism of Nitrogen, Calcium and Phosphorus in Human Adults on a Poor Vegetarian Diet Containing Ragi (Eleusine coracana). V. Subrahmanyan, M. Narayanarao, G. Ramarao, and M. Swaminathan. *Brit. J. Nutrition* 9: 350, 1955.

Ragi is a grain of considerable importance in the dietary of millions of people who inhabit the Deccan Plateau of India. The grain is very small and has a diameter of only 1 to 1.5 cm. In view of its small size and the difficulty of separating the seed coat, it is usually cleaned and ground as a whole for use as flour. The flour is generally cooked to a paste with the minimum amount of water and the paste rolled into balls which are eaten with soups and cooked vegetables. The working classes prefer ragi to softer grains like rice, because of its cheapness and sustaining qualities.

The intake and absorption of nitrogen, calcium, and phosphorus were studied in 8 men on a poor vegetarian diet based on ragi. The composition of the diet was similar to that consumed by the poorer people in Mysore State. All the experimental subjects maintained a positive nitrogen balance. However, the excretion of nitrogen in feces was very high, the apparent digestibility of the proteins being only 50 per cent. All the subjects maintained positive calcium and phosphorus balances. The average intake of phytate phosphorus was 80 per cent of the total phosphorus ingested. About 85 per cent of the ingested phytate was found to have been hydrolyzed.

The results of growth experiments on albino rats have shown that the total nutritive value of a poor vegetarian diet containing ragi is higher than that of similar diets containing rice or kaffir corn and almost equal to that of a wheat diet. Such information should be obtained on human subjects before conclusions can be drawn on the value of ragi as a protein source for the inhabitants of the Deccan Plateau of India, particularly because of the low protein content (6.8 per cent) and very low digestibility of this cereal grain.—B. SURE

Postoperative Course Following Total Right Hepatic Lobectomy. A. H. Islami, G. T. Pack, T. R. Miller, P. Vanamee, H. T. Randall, and K. E. Roberts. *Surgery* 39: 551, 1956.

A brief account of the clinical course of four patients who underwent total right hepatectomy for malignant tumors involving the liver is presented. One patient died two and a half years after operation; three were still living and apparently well one and one-half years postoperatively. Certain biochemical measurements made during the preoperative and early postoperative period (2 to 3 weeks) are reported. Following operation, there was a prompt, but transient, alteration of certain liver function tests. There was a rise in serum bilirubin in all, none of whom were jaundiced in the immediate preoperative period; in three patients, the jaundice had cleared by the twenty-first day. Plasma albumin concentrations fell in all; plasma globulin, in two. Changes in most of the plasma electrolytes measured were minimal (sodium, potassium, chloride, and calcium); in three, there was a transient fall in plasma phosphate. Plasma carbon dioxide and pH were normal in all. Blood ammonia levels did not rise significantly in these patients. (No specific information on dietary intakes is given.) Tolerance to intravenous ammonium acetate (as judged by blood ammonia levels and EKG changes) was measured in three anesthetized dogs in the first day following 70 per cent hepatectomy; their tolerance was similar to that observed in two anesthetized, but otherwise normal, dogs.—S. M. LEVENSON

Further Clinical Observations in Galactosemia: A Possible Mode of Production. G. Komrower. *Am. J. Dis. Child.* 90: 512, 1955 (Soc. Trans.).

The finding of a marked aminoaciduria in untreated cases of galactosemia has been repeated in two other children; this was associated with a marked proteinuria. In all children both conditions returned to normal when a lactose- or galactose-free diet was introduced. These conditions have been reproduced in one child now aged 3 years.

A marked acidosis has been demonstrated in two infants on galactose or milk diets; this also disappeared when the special diet was recommended. Investigations suggest that this is a renal acidosis similar to that found in hyperchloremic acidosis and that the condition becomes increasingly difficult to reproduce as the infant develops.

It is thought that these findings are due to the presence in the cells of galactose-1-phosphate. It is suggested that there is an interference with cell metabolism and the increase in the amount of galactose-1-phosphate may mean a reduction in the amount of glucose available in the cell and so diminish the amount of energy available for the cell metabolism.—J. N. ETTELDORF

A Study of Anabolic Effect of Nortestosterone Cyclopentylpropionate and Its Effect upon the Excretion of N^{15} Administered Intravenously as N^{15} Glycine. G. Hollifield, K. R. Crispell, and W. Parson. *Metabolism* 5: 165, 1956.

Nitrogen balance determinations obtained on three subjects following an intramuscular injection of 300 mg per cent of nortestosterone cyclopentylpropionate (NTP) revealed a duration of positive balance of 29, 24, and 30 days, respectively. The duration of positive nitrogen balance following testosterone propionate (TP) was approximately one-half that seen with NTP in one patient. A greater excretion of 17-ketosteroids was noted with TP than with NTP. The latter agent caused a marked decrease in the excretion of N^{15} glycine, suggesting that more nitrogen was made available for protein synthesis.—C. R. SHUMAN